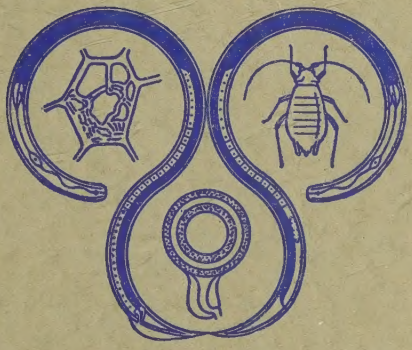
ENTOMOLOGY LIBRARY
JUL 1961
RIAL Eu.86
EPARATE

TIJDSCHRIFT OVER PLANTEZIEKTEN

JAARGANG 67 - 1961

1e AFLEVERING



Uitgave van de

NEDERLANDSE PLANTEZIEKTENKUNDIGE VERENIGING

Onder redactie van

Dr. J. A. VON ARX, Mej. H. L. G. DE BRUYN,
Mevr. Ir. M. POST-BAKKER, Prof. Dr. J. P. H. VAN DER WANT
en Dr. H. H. EVENHUIS (Secretaris)

Redactie-adres: Instituut voor Plantenziektenkundig Onderzoek,
Binnenhaven 4*a*, Wageningen

BESTUUR
VAN DE NEDERLANDSE PLANTEZIEKTENKUNDIGE VERENIGING

Dr. P. M. L. Tammes, Wageningen, *Voorzitter*
Ir. J. A. J. Veenenbos, Plantenziektenkundige Dienst, Wageningen, *Secretaris*
Dr. A. P. Kole, Lab. v. Fytopathologie, Binnenhaven 4, Wageningen, *1e Penningmeester*
Postrekening no. 92 31 65 ten name van Penningmeester van de Nederlandse Plantenziektenkundige Vereniging te Wageningen
Mevr. Ir. M. Post-Bakker, Lawickse Allee 80, Wageningen, *2e Penningmeesteresse*
Ir. H. J. de Bruin, Wageningen
H. P. Dijksterhuis, Garrelsweer
Dr. J. C. s' Jacob, Wageningen
Ir. J. Walrave, Wageningen
Prof. Dr. J. P. H. van der Want, Lisse

Lidmaatschap Ned. Planteziektenkundige Vereniging

Nederland, Suriname en de Nederlandse Antillen f 7,50, België f 7,50, Buitenland f 8,50. Leden ontvangen het tijdschrift gratis.

Opzegging van het lidmaatschap moet vóór 1 december van het lopende jaar geschieden, anders is de contributie voor het volgende jaar verschuldigd.

Abonnement Tijdschrift

Nederland, Suriname en de Nederlandse Antillen f 10,—, België f 10,—, Buitenland f 13,50. Betaling op postrekening no. 92 31 65 t.n.v. Penningmeester Ned. Planteziektenkundige Vereniging te Wageningen.

Advertentiën

Prijzen per plaatsing: ⅛ pag. f 12,50; ¼ pag. f 20,—; ½ pag. f 30,—; 1 pag. f 55,—.
Bij 3 maal plaatsen van dezelfde advertentie 10 procent korting.
Bij 6 maal plaatsen van dezelfde advertentie 20 procent korting.

Voor plaatsing wende men zich tot de secretaris der Redactie-Commissie, p.a. Binnenhaven 4*a*, Wageningen, tel. 2151, of tot H. Veenman & Zonen N.V., Wageningen, tel. 2242.

INHOUD

HOOFDARTIKELEN

G. H. BOEREMA – An underground attack of the rust *Uromyces colchici* on *Colchicum* in the Netherlands. *Met een samenvatting: Een in Nederland waargenomen ondergrondse aantasting door de roest* Uromyces colchici *bij* Colchicum 1
A. KAARS SIJPESTEIJN – New developments in the systemic combat of fungal diseases of plants. *Met een samenvatting: Nieuwe ontwikkelingen in de bestrijding van schimmelziekten bij planten met systemisch werkzame middelen* 11
G. J. M. A. GORTER – The toxicity of some fungicides to conidia of *Gloeosporium fructigenum* f. *chromogenum*. *Met een samenvatting: De toxiciteit van enkele fungiciden voor de conidiën van* Gloeosporium fructigenum *f.* chromogenum 21
J. DEKKER – Systemic activity of procaine hydrochloride on powdery mildew. *Met een samenvatting: Systemische werking van procaïne-hydrochloride tegen echte meeldauw* . 25
W. HELLE en W. OUDSHOORN – Multiresistentie van de bonespintmijt *(Tetranychus urticae)* in Aalsmeer. *With a summary: Multi-resistance in the two-spotted spider mite* (Tetranychus urticae) *at Aalsmeer* 28

BOEKBESPREKING

H. BRAUN & E. RIEHM – Krankheiten und Schädlinge der Kulturpflanzen. Achte, neubearbeite Auflage (E.) 32

TIJDSCHRIFT OVER PLANTEZIEKTEN

Onder redactie van Dr. J. A. VON ARX; Mej. H. L. G. DE BRUYN;
Mevr. Ir. M. POST-BAKKER; Prof. Dr. J. P. H. VAN DER WANT
en Dr. H. H. EVENHUIS (secretaris)

Redactie-adres:
Instituut voor Plantenziektenkundig Onderzoek, Binnenhaven 4a, Wageningen

Drukkers-Uitgevers: H. Veenman & Zonen N.V. – Wageningen

67e JAARGANG (1961)

AN UNDERGROUND ATTACK OF THE RUST *UROMYCES COLCHICI* ON *COLCHICUM* IN THE NETHERLANDS[1]

Met een samenvatting: *Een in Nederland waargenomen ondergrondse aantasting door de roest* Uromyces colchici *bij* Colchicum

BY

G. H. BOEREMA
Plantenziektenkundige Dienst, Wageningen

INTRODUCTION

Colchium rust in the Netherlands was first brought to our attention in 1955 by a representative of the Plant Quarantine Division, United States Department of Agriculture. The finding was made by Mr. N. G. SANTACROCE, an official of that Division, who has been working in The Netherlands by cooperative agreement on the pre-shipment clearance of flower bulbs. The first *Colchicum* found to be infected by him was the *Colchicum* hybrid "Violet Queen". The rust was found on the dry, outer tunic of the corm.

Since that time this disease has been intercepted several times on the same hybrid. Indications are that the disease has become widespread in the hybrid "Violet Queen". The disease has also been discovered on growing plants in the field.

Mr. N. G. SANTACROCE (unpublished) reports that this rust has also been found on the dry tunics of *Colchicum bornmuelleri* Freyn and the *Colchicum* hybrids "Autumn Queen" and "The Giant".

By careful examination of the material it appears that this curious rust, ocurring for the most part on the subterranean parts of *Colchicum* in The Netherlands, is identical with *Uromyces colchici* Massee. This species is reputed to be of great rarity; it has been found only on two other occasions in England.

HISTORY OF THE ABOVE-GROUND ATTACK OF *UROMYCES COLCHICI*

The Fungus

Uromyces colchici was first described by MASSEE in 1892: "Sporeclusters

[1] Accepted for publication 1 Nov., 1960.

numerous, large, elliptical, sometimes, circinating blackish-brown, occurring on both surfaces of the leaf; teleutospores broadly elliptical or subglobose, apex slightly prominent, and pierced by a single germspore, epispore about 24 μ thick, smooth, bright-brown, 28–38 × 21–28 μ; pedicel colourless, persistent, uniformly attenuated towards the base, 70–80 × 5–6 μ.
On *Colchicum spectabilis*, Kew Gardens."

This description has been copied into the textbooks of COOKE (1906) and GROVE (1913). GROVE (1913) investigated the original material of MASSEE and corrected the description of the teleutospores as follows:

"Spores subglobose to ovate, rounded above, with a broad flat hyaline papilla, smooth, pale-brown, 28–40 × 20–28 μ; epispore 3–3½ μ thick; pedicels hyaline, rather long, but very deciduous."

The type material of *U. colchici* is deposited at the Herbaria of the New York Botanical Garden, and of the Royal Botanical Gardens at Kew where three leaf-fragments with teleutosori remain. From our investigation of the latter, it is established that GROVE's (1913) description of the teleutospores of *U. colchici* (fig. 1) is the most accurate. It must be noted, however, that the spores are not smooth, but instead are covered with regularly arranged small warts. This is clearly seen when one examines the teleutospores dry or in an air-bubble (fig. 1C).

WILSON & BISBY (1954) also investigated the material at the Kew Herbarium and concluded that *U. colchici* bore great resemblance to *Uromyces gageae* Beck. A great deal of controversy exists concerning the identity of *U. gageae*. Some authors believe that this rust is the same as *Uromyces ornithogali* Lév. (e.g. GUYOT, 1951). Others infer that *U. gageae* and *U. ornithogali* are not identical at all (e.g. HYLANDER, JØRSTAD & NANNFELDT (1953) who indicate *U. ornithogali* with the synonym *Uromyces acutatus* Fuck.[1] Nevertheless, it is absolutely certain that *U. colchici* is not identical to the known rusts occurring on *Gagea* spp. and *Ornithogalum* spp. The teleutospores of *U. colchici* are very characteristic and differ from those of *U. gageae* and *U. ornithogali*. For instance, the teleutospore of *U. colchici* has an obviously broad and flat papilla (vide fig. 1 and GROVE, 1913). The spores of the species *U. gageae* as well as those of *U. ornithogali* have papillae which are narrow and long. Furthermore, the epispore of *U. colchici* is considerably thicker than that of either *U. gageae* or *U. ornithogali*. It is by this characteristic papilla that *U. colchici* can be distinctly distinguished from all other *Uromyces* species described on bulbous and rhizomatous plant species in the families Amaryllidaceae, Iridaceae and Liliaceae.

From the observations of MASSEE (1892, 1899) it may be said that *U. colchici*

[1] The background of this controversy was a dispute as to whether there are or are not present little warts on the teleutospores. In *U. gageae* the teleutospores are supposed to be smooth and decidedly verruculose in the case of *U. ornithogali*. In our investigations of *U. gageae* and *U. ornithogali* from material obtained from the herbaria at Kew, Leyden and Groningen it would appear that there is actually a question of two types. In some of the exsiccates examined, the dark-brown teleutospores showed conspicuously clear warts, while most of the exsiccates, among which was the original material of BECK, the teleutospores which were coloured pale-brown were quite smooth. However, from a casual examination, it is found that the spores of latter type (pale-brown) are also covered with little warts! This can be seen more clearly when the spores are observed dry or in an air-bubble.

is a rust which does not change of host-plant (autoecious), forming teleutospores solely, which must hibernate before germination (micro-type)[1].

The above-ground symptoms as observed by MASSEE

MASSEE reported the extremely large elliptical teleutosori (5–8 × 3–5 mm) on both sides of *Colchicum*-leaves (compare fig. 2A).

The sorus on the leaf-fragment from the herbarium at Kew which we examined measured 7 × 3.5 mm (fig. 2B). These sporeclusters remain covered a long time by the epidermis, and are often found to be circinating on the leaf-blade (fig. 2A). MASSEE found them more commonly on the lowest leaf (first formed leaf). The sori appear to develop first on the leaf-sheath and later occurring on the leaf-blade as well. On the higher (later developed) leaves only some sporadically scattered teleutosori were found, always developing much later, which suggests an upward extension of the mycelium (MASSEE, 1892).

Host plants and localities of the above-ground infection

The host-plant mentioned in the original description of MASSEE in 1892 as *Colchicum spectabilis* has not been described elsewhere. The specific identification of this host plant, apparently, appears to be a mistake, because later on MASSEE (1899, 1915), COOKE (1906), and GROVE (1913) continually write about *Colchicum speciosum* Stev. It is from this latter species, which has its origin in Asia Minor and the Caucasus, that many *Colchicum* hybrids (BOOM & RUYS, 1950) have been derived. For two years, MASSEE (1892) found the rust exclusively on this species of *Colchicum*, which was evidently so susceptible that all plants on the plot were infected with the rust. In the third year, MASSEE (1899, 1915) observed that the rust had infected the adjacent plants of *Colchicum autumnalis* L. and "*Colchicum bavaricum*". The latter species has never been described as such; however, WILSON & BISBY (1954) have concluded that *C. bavaricum* is a synonym of *C. variegatum* L. which also comes from Asia Minor. MOORE (1959) has adopted this interpretation. Following the observations of MASSEE, *U. colchici* was found once more in Yorkshire, England (MASON & GRAINGER, 1937) on an unidentified species of *Colchicum*.

GUYOT (1951) points out that UNGER (1833) mentioned the presence of an *Uredo* sp. on *Colchicum autumnale* L. in Austria. This might have been the same rust as *U. colchici*, since formerly many species of *Uromyces* were listed as *Uredo* (compare GÄUMANN, 1959[2]). Unfortunately UNGER did not give a description, consequently the relationship of this fungus cannot be established.

Besides the reports mentioned above, we know of no other observations on the occurrence of this fungus on the above-ground parts of *Colchicum*. Therefore the remark of GÄUMANN (1959) "Verbreitungsgebiet sporadisch durch ganz Europa" (occurring sporadically in all Europe) is only hypothetical.

[1] During the German expedition to the Himalaya in 1937, a rust with an aecidium stage was found on the leaves of *Colchicum aureum* Bak., which was described by ULBRICHT as *Aecidium colchici – aurei* Ulbr. (See MOORE, 1949 as well). In France, a rust with an uredial stage very similar to an aecidium had been found on *Colchicum autumnale* L., which was described as *Uredo colchici – autumnalis* Guyot & Massenot (GUYOT, 1958).
It is, however, certain that these stages of rusts do not belong to the life-cycle of *Uromyces colchici* Massee.

[2] UNGER (1833) did not use the binomen *Uredo colchici* as GÄUMANN (1959) erroneously concluded from GUYOT (1951).

Description of the underground attack

Hostplants

The *Colchicum* hybrid "Violet Queen" produces pretty, dark-purple flowers with a white base. Corms of this variety have been found infected with *Uromyces colchici* since 1955 in the Netherlands. The parental origin of this hybrid, already distinguished as "first class" in 1927 (Anonymus, 1956) can no longer be traced. Undoubtedly, however, it appears to be an offspring of the rose-purple flowering *C. speciosum* Stev. (Boom & Ruys, 1950), the species on which *U. colchici* was originally found. This is also true of the *Colchicum* hybrids "Autumn Queen" and "The Giant" which are also reported as being attacked by *U. colchici*. Both hybrids have been in cultivation for a long time (Anonymus, 1956). *Colchicum bornmuelleri* Freyn has also been observed infected by this rust. Both *C. bornmuelleri* and *C. speciosum* came originally from Asia Minor.

Symptoms of the disease

The teleutosori of *U. colchici* were found on the corm-tunic of the above-named *Colchicum*s. This tunic is the well developed sheath of the lowest leaf. In most cases, the teleutosori were found exclusively on the innerside of the tunic (compare fig. 3). In some cases, however, teleutosori were observed on the outer side of the tunic and located at the same position on the tunic as the inner teleutosori.

Infections of the tunic are usually confined to one side of the corm. Usually, this was a "side" of the characteristic bilateral symmetrical corm (compare figs. 3A, 5, 6). In a few cases, the infection was not quite so obviously one-sided and the teleutosori were also found on the flattened, extended side of the corm (fig. 3C).

From the specimens examined, teleutosori were also found on the upper part of the corm-tunic, that is on that part of the tunic which encloses the (old) stem. Coincidentally, it is found that sometimes on the growing plant, spots of the rust can be seen on the blanched part of the sheath of the lowest leaf. Tunic (outer covering of corm) infections are naturally visible only after the corms have been dug from the soil. Teleutosori have never been observed on the green parts of the leaf-sheats nor on the leaf-blades of the growing plants in The Netherlands. Sometimes, *Colchicum* plants having infected corms, showed conspicuous yellow spots on the leaf-blade of the lowest leaf. All other above-ground parts of the plant and the fleshy part of the corm showed no signs of the disease. It would appear, therefore, that infection remains restricted to the tunic (including leaf-sheath).

Teleutosori can be observed most clearly immediately after digging, before the tunic becomes dry and brown. It is at this time, when the tunic is still light coloured, that there is the greatest contrast with the dark-brown soreclusters. When teleutosori are formed on the inner side only, the outer symptoms of the tunic will show as somewhat swollen, brown spots (fig. 3A, B). By carefully separating the infected part of the tunic from the flesh, there is observed an excellent view of the soreclusters formed on the inner side of the tunic (fig. 3D, left). In doing so, spores of the rust usually remain adhering to the white flesh of the corm giving the appearance of a brownish-black powder (fig. 3D, right). When the teleutospores become mature, the tunic becomes broken or torn in the center of the infected spot (fig. 3C, D).

It is remarkable, that the teleutosori, which are at first covered by the epidermis (fig. 4), are often arranged in concentric rings (fig. 3D). In most cases, it could be established that infection had developed along the vertically running main veins (fig. 3B, 4).

The dimensions of the teleutosori are 1–3 mm by 1–8 mm, but often there is no definate separation between sori. The teleutospores were completely identical in detail to those examined from material of MASSEE of the Kew–Herbarium (fig. 1).

It was noted that when the tunic becomes brown and dead which is usually at the time of late harvest or later, the teleutosori become less prominent. The tunic then has nearly the same colour as the granular masses of teleutospores (fig. 5A). As a result of the development of the teleutosori on the inner side of the tunic, it becomes lossened from the flesh at these spots. As result of this separation, rather large segments of the infected tunic remain in the ground during corm harvesting (figs. 5B, 6). The brownish-black powdery spores on the white corm-flesh are rather conspicuous at this time (fig. 6).

In so far as the infected tunic has not yet come off, characteristic, dark-brown, concentric rings may often be seen in diseased spots (fig. 5B).

In experiments with diseased corms, completely healthy flowers developed in the field with no evidence of infection. All leaves were also quite healthy. The newly-formed corms were in most cases again infected. In a few cases, the newly formed corms, produced by planting diseased corm material, showed no signs of infection.

DISCUSSION

The phenomena of the underground attack

We are confronted here with a curious fact that a rust has been found on the underground tunic of *Colchicum* hybrids and the species *Colchicum bornmuelleri* in The Netherlands, which in the last century had been found incidentally on the leaves of *Colchicum speciosum* and other *Colchicum* species.

This infection may be characterized as being "an underground infection", as the disease develops on that part of the plant which is in the soil, although, some of the teleutosori may develop on the part of the tunic which encloses the stem above the soil level.

This unusual rust attacking the subterranean parts of a plant, is not the only case known, for a quite similar rust attacking the underground parts of the plant has been regularly found on *Crocus* hybrids in The Netherlands since 1956 (BOEREMA, 1959). In the latter case we are concerned with the rust, *Uromyces croci* Pass., a species found regularly on the leaves of different species of *Crocus* in South and South-East Europe, dating back to 1826. A major difference between the underground infections of *Crocus* and *Colchicum* is, that in the case of *Crocus* the fungus attacks the tunic and the fleshy part of the corm, while in *Colchicum* only the tunic is affected. Consequently *U. croci* causes more damage on the whole than *U. colchici* on affected corms.

From the facts established here, there follows the assumption that we are dealing with two rusts which by adaptation have become able to attack the underground parts of *Colchicum* and *Crocus* in The Netherlands. This adaptation is probably on account of the application of special cultivation methods of *Colchicum* and *Crocus* which have been in effect for many decennia. The possibili-

ties for the above-ground life-cycle of these rusts are, in effect, practically reduced to zero by the combination of the practice of harvesting the corms of these plants every year and multi-annual crop rotation. In other words, these rusts can only maintain themselves by adaptation to the underground parts of these plants. Therefore, in these underground infections one could probably speak of genetically "adapted" strains. Naturally, this hypothesis will still need to be proven.

The corm-tunic of *Colchicum*, as has been already stated above when discussing symptoms of the disease, is no more than the well developed leaf-sheath of the lowest leaf. Consequently, the lowest leaf and the tunic are homologous. Since MASSEE (1892, 1899) observed the infection on the leaf-sheath and leaf-blade of the lowest leaf, it can be said that there is very little difference between the above-ground infection found by him and the underground infection occurring in The Netherlands. Anatomically, the tunic on *Colchicum* does not differ greatly from an ordinary leaf. The only real difference is that the tunic lacks chlorophyll. If this is really a question of genetical adaptation in the case of the underground infection, the presence or absence of chlorophyll is perhaps also essential for the respective strains found above-ground and underground. Significantly it is typical in the case of the disease observed in The Netherlands that, whenever teleutosori are found on the above-ground parts, they are found only in the leaf-sheath that is devoid of chlorophyll. In this connection, further investigations referring to the small, yellow spots which are sometimes found on the leaf-blade of the lowest leaf in underground infections may give indications. With the infection of leaves by rust-fungi, chlorophyll is generally only important as an indirect provider of a nutrient-source for the fungus (compare HORSFALL & DIMOND, 1959).

A striking feature of the underground infection is that the teleutosori are for the most part found on the inner side of the tunic. While MASSEE found the teleutosori on both sides of the leaves, they were apparently found especially on the outer side of the leaf-sheath (fig. 2). Also, in the case of the underground infection of *Crocus* by *Uromyces croci*, the teleutosori are developed mainly on the inner side of the tunic. These are possible logical consequences in the deviating way of life concerning underground infections, because the formation and development of the new plant parts, to become infected by the teleutospores, are produced on the inner side of the old tunic.

The infection cycle of the above-ground and underground attack

From the figures produced by MASSEE (1899, 1915) and COOKE (1906) on leaf infection (compare fig. 2A), it would appear that in above-ground infection the teleutosori were formed in many cases on the veins of the leaf. This was often found to be true in the case of underground infections also (fig. 3A, B). By means of microtome sections (fig. 4), it was further established that the mycelium of the rust in underground infection grows rank in the cells of the vascular bundles (intracellular) and that it communicates from there with the teleutosori formed under the epidermis via whole mycelium strands between the big parenchyma cells of the tunic (intercellular). It seems plausible, therefore, that the extension of the infection, in both cases, has taken place via the veins especially. This is in accordance with MASSEE's observation that the above-ground infection nearly always begins on the leaf-sheath and gradually becomes extended over the leaf-

blade. Therefore, it may be explained that in underground attack observed id The Netherlands, where infection generally occurs underground, the teleutosori will also develop eventually on the above-ground part of the tunic, that is, on the white part of the sheath of the lowest leaf. The formation of teleutosori on the higher, green leaf parts is then possibly prevented by the presence of chlorophyll (see above).

In addition, in view of the concentric arrangement of the teleutosori commonly found in the above-ground infection reported by MASSEE (1899, 1915) (cf. fig. 2), and in the underground infections (fig. 3D), there is also question of a concentrical extension of the mycelium. In the case of the underground infection, this concentrical extension is exceedingly conspicuous on account of the dark-brown, concentric rings in the dead, dry tissue (fig. 5B).

From MASSEE's descriptions, it may be derived that in the case of above-ground attacks, secondary infections did not occur in the same season. Consequently, all teleutosori on the plants had developed in the above-mentioned systemic way from a primary infection in the spring. This is further explained by the absence of other spore-stages and by the fact that MASSEE (1899) established that teleutospores can germinate only after hibernating (autoecious-microcycle).

GROVE (1913) describes the life-cycle of the above-ground infection as follows:

> "The teleutospores remain on the dead leaves and germinate in the following spring, so that if *Colchicum* is again planted in the same ground or allowed to remain there, it is liable to contract the disease year after year".

Concerning the underground infection on *Colchicum*, the same principle may be assumed to hold. The new infections probably take place in spring by basidiospores from the germinating teleutospores coming from the old, diseased tunics. In this way, the new corm and the small split-corms formed within the old tunics can become infected. In addition, the anatomy of the underground parts of *Colchicum* is such that this infection may take place on both "sides" and on the flattened part of the newly-formed corms. At these places, the old tunic is directly bounded by the membranous tunic of the new corms, while the bulging and rounded part of the new corms is "protected" by the corm-flesh of the old one. This may explain why teleutosori are especially found on one "side" only of the new corm, although occasionally they are found on the flattened side also.

When healthy corms are planted in a soil in which diseased tunic fragments are present, the new corms may become infected by the germinating spores in spring. Infection of newly-formed corms may take place naturally by the spread of spores in the sori which are sometimes found at ground level.

That sometimes newly-formed corms had not become infected when produced from diseased planting material, may be explained by a period of storage, unfavourable to spore conservations.

AMPLIFIED DESCRIPTION OF *UROMYCES COLCHICI*

Uromyces colchici Massee 1892 (Grevillea 21,6) char. ampl. Autoecious – microcycle.

Two different types of attack:

A. Leaf infection (Massee, 1892, 1899, 1915, Grove, 1913)

Teleutosori distributed on the leaf-sheath and on both sides of the leaf-blade, most abundant on the lowest leaf and always commencing at the base of the sheath; sori on the sheath, large, elliptical, 5–8 × 3–5 mm, often confluent; on the leaf-blade as a rule smaller and often arranged in circular groups; in both cases for a long time covered by the epidermis which cracks at length, subpulverulent, brown.

B. Tunic infection

Teleutosori on the inner side and incidentically on the outside of the corm-tunic; sometimes also on the connecting part of the leaf-sheath of the lowest leaf which does not contain chlorophyll; sori on the tunic most often on one side of the typically two-sided symmetrical corm, but sometimes on the flattened and elongated side of the corm; varying greatly in dimensions, 1–8 × 1–4 mm, often arranged in concentric rings without distinct borders between the sori, at first covered by the epidermis, which later becomes broken.

Teleutospores subglobose to ovoid, round above, with a broad flat hyaline papilla, regularly covered with small warts, palebrown, 28–40 × 20–28 μ; epispore 3–3½ μ thick; pedicels hyaline, uniformly attenuated toward the base, 70–80 × 5–6 μ, very deciduous.

Leaf infection found on *Colchicum speciosum* Stev. (= *C. spectabilis* Auct.), *Colchicum autumnale* L. and *Colchicum bavaricum* Auct. (= *C. variegatum* L.?), Kew Gardens, England (Massee 1892, 1899, 1915; Wilson & Bisby, 1954) and on *Colchicum* spec., Yorkshire (Mason & Grainger, 1937).

Tunic infections found on the *Colchicum* hybrids "Violet Queen", "Autumn Queen", "The Giant" and *Colchicum bornmuelleri* Freyn, bulb fields in The Netherlands.

Herbarium material with the tunic infection has been deposited in the "Rijksherbarium", Leyden, The Netherlands and The Herbarium of the Royal Botanic Gardens, Kew, England.

Summary

1. Underground infection of *Colchicum* by the rust *Uromyces colchici* Massee has been regularly observed in The Netherlands since 1955.
2. A review of the literature is given. The disease is reputed as being one of extreme rarity. Symptoms of the diseased corms are extensively described here.
3. In the discussion, an analysis and comparison has been made between the underground corm infection found in The Netherlands and the above-ground infection observed earlier.
4. Finally an amplified description of *Uromyces colchici* is given.

Samenvatting

Sinds 1955 wordt bij de in Nederland gekweekte *Colchicum*-hybride „Violet Queen" regelmatig een roestaantasting van de knolrok geconstateerd. Incidenteel werd deze als „ondergronds" te karakteriseren aantasting ook vastgesteld bij *Colchicum bornmuelleri* Freyn en de *Colchicum* hybriden „Autumn Queen" en „The Giant".

De betreffende roest bleek identiek te zijn met *Uromyces colchici* Massee (1892). Deze niet-waardplantwisselende roest, die uitsluitend teleutosporen vormt (fig. 1), staat als zeer zeldzaam te boek. Bovengrondse bladaantastingen

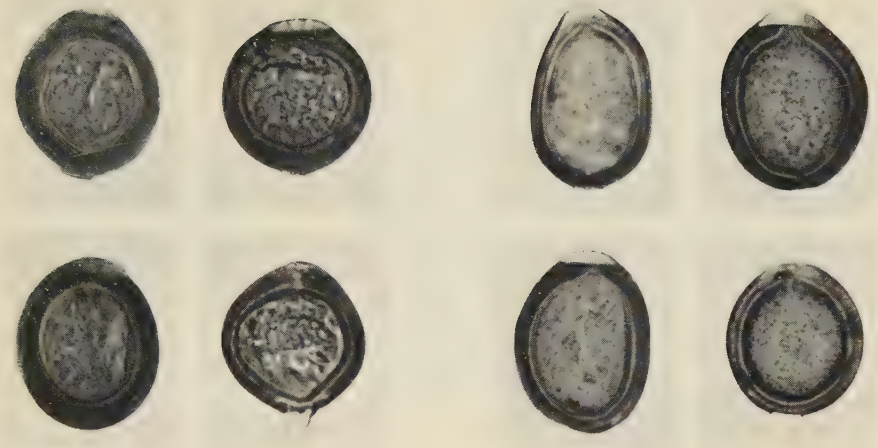


Fig. 1A

Fig. 1B

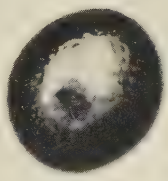


Fig. 1C

FIG. 1. Teleutospores of *Uromyces colchici* Massee. Magnification × 640.

A. Optical section. Spores from the leaf of *Colchicum speciosum* Stev. Material of MASSEE, 1892. Herbarium Royal Botanic Gardens Kew.

B. Optical Section. Spores from the corm-tunic of the *Colchicum* hybrid "Violet Queen" 1959.

C. Lateral view in air-bubble. Spore from the leaf of *Colchicum speciosum* Stev. Material of MASSEE, 1892. Herbarium Royal Botanic Gardens Kew.

Teleutosporen van Uromyces colchici *Massee. Vergroting 640* ×.

A. *Optische doorsnede. Sporen van blad van* Colchicum speciosum *Stev. Materiaal van Massee, 1892. Herbarium Royal Botanic Gardens Kew.*

B. *Optische doorsnede. Sporen van knolrok van* Colchicum-*hybride „Violet Queen" 1959.*

C. *Zijaanzicht in luchtbel. Spore van blad van* Colchicum speciosum *Stev. Materiaal van Massee, 1892. Herbarium Royal Botanic Gardens Kew.*

Fig. 85. — *Uromyces colchici*. 1, portion of a diseased *Colchicum* leaf; 2, teleutospores, one of which has germinated and produced a promycelium bearing three secondary spores. Fig. 1 reduced, Fig. 2 mag.

Fig. 2A

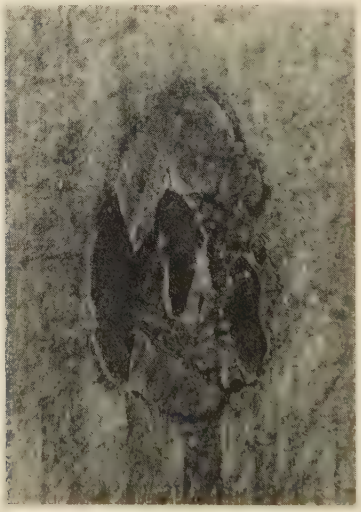


Fig. 2B

FIG. 2A. A reproduction from MASSEE: Diseases of cultivated plants and trees (1915).

Tekening van het door Massee waargenomen bovengrondse ziektebeeld van Uromyces colchici *Massee, gecombineerd met een afbeelding van twee teleutosporen, waarvan één is gekiemd. Reproduktie uit: „Diseases of cultivated plants and trees", Massee, 1915.*

B. Photograph of a teleutosorus of *Uromyces colchici* Massee on a leaf fragment of *Colchicum speciosum* Stev. Magnification × 6.5. Material of MASSEE, 1892. Herbarium Royal Botanic Gardens Kew.

Foto van een teleutosorus van Uromyces colchici Massee *op een bladfragment van* Colchicum speciosum *Stev. Vergroting 6,5 ×. Materiaal van Massee, 1892. Herbarium Royal Botanic Gardens Kew.*

Fig. 3A

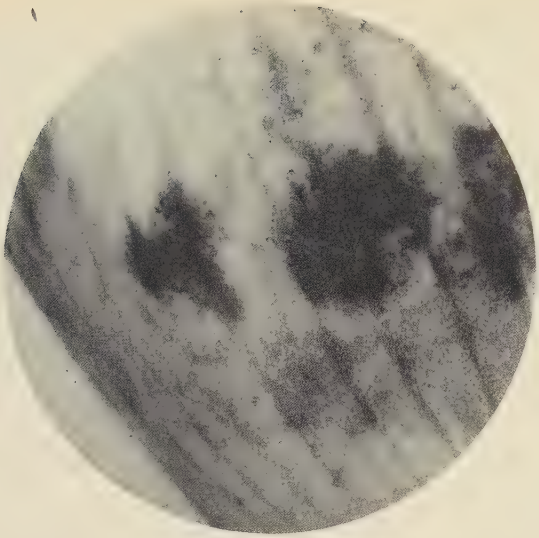


Fig. 3B

Fig. 3C

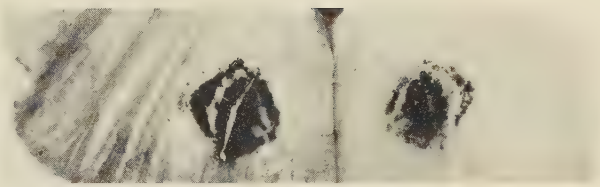


Fig. 3D

FIG. 3. Early dug plants of the *Colchicum* hybrid "Violet Queen" attacked by *Uromyces colchici* Massee.

A. Early stage: The slightly protuding brown spots indicate the places where teleutosori have developed on the inner-side of the tunic.
B. Detail of these brown spots: Position on the veins is clearly visible.
C. Advanced stage: Cracked tunic in the area in which teleutosori are formed.
D. Left: Exposed inner side of the diseased tunic showing mass of teleutosori in concentric rings. Right: Imprint of diseased rust spot on the white-corm-flesh made by loosened, dark-brown teleutospores.

Vroeg gerooide planten van de Colchicum-*hybride „Violet Queen", aangetast door* Uromyces colchici *Massee.*

A. *Vroeg stadium: De plaatsen, waar aan de binnenzijde van de rok teleutosori zijn gevormd, zijn te herkennen als iets verheven, bruine vlekjes.*
B. *Detail van deze bruine vlekjes: Het is goed te zien dat ze zich op de nerven bevinden.*
C. *Later stadium: De rok is in het centrum van de aantasting opengescheurd.*
D. *Links: Aan de binnenzijde van de los geprepareerde rok zijn de in concentrische ringen gerangschikte teleutosori zeer opvallend.*
Rechts: De losgelaten teleutosporen vormen een afdruk van de aantasting op het witte knolvlees.

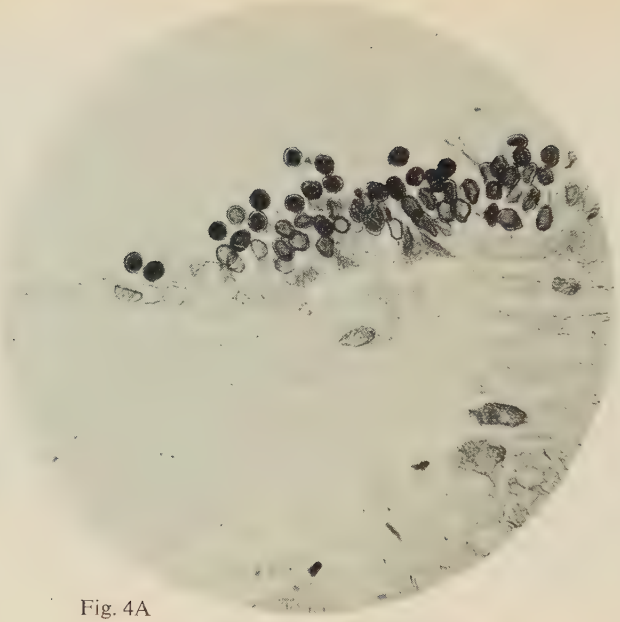


Fig. 4A

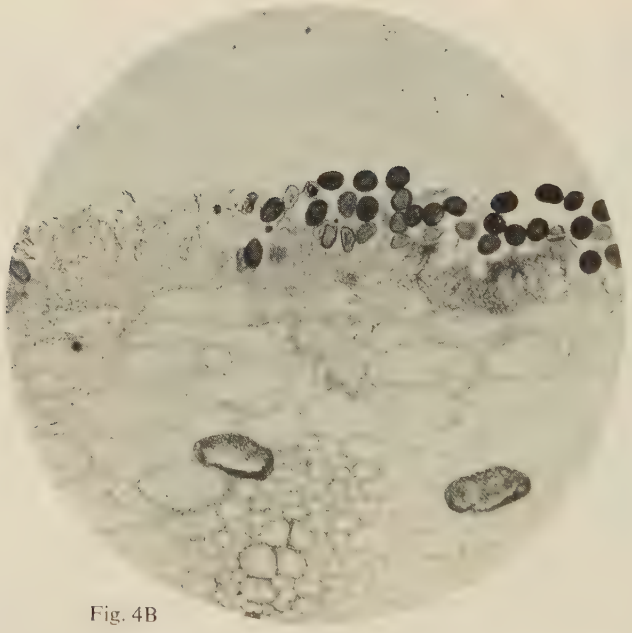


Fig. 4B

FIG. 4. Transverse microtome sections of the tunic showing parts of a teleutosorus of *Uromyces colchici* Massee. Magnification × 130.
Photograph A: Part of the remaining epidermis at left. On both photographs the mycelium is visible among the large parenchymacells (intercellular) and in the cells of the vascular bundle (intracellular) of the photograph B.

Dwarse microtoom-coupes van een door Uromyces colchici *Massee aangetaste knolrok. Vergroting 130 ×. De epidermis is alleen op foto A nog te zien (links); boven de teleutosori is deze niet meer aanwezig. Op beide foto's ziet men tussen de grote parenchymatische cellen het mycelium van de roest (intercellulair), terwijl op foto B te zien is dat dit mycelium ook woekert in de cellen van de vaatbundel (intracellulair).*

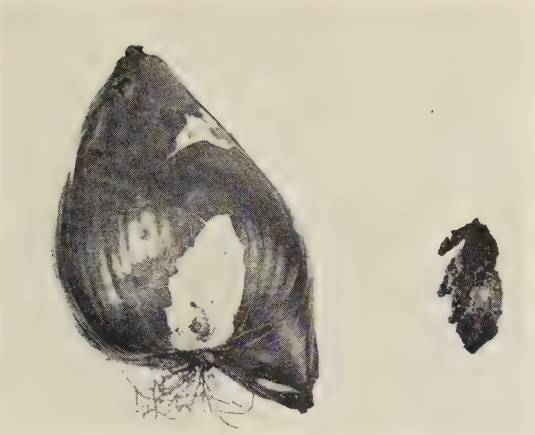


Fig. 5A

Fig. 5B

FIG. 5. Mature corms of the *Colchicum* hybrid "Violet Queen" infected by *Uromyces colchici* Massee.

A. A piece of the infected tunic removed to show the ripened teleutosori from the inner side.

B. Still visible dark-brown concentric rings in the diseased tunic.

Afgerijpte knollen van de Colchicum-*hybride „Violet Queen", aangetast door* Uromyces colchici *Massee.*

A. *Een deel van de aangetaste rok is verwijderd om de rijpe teleutosori aan de binnenzijde te laten zien.*

B. *Op het nog aanwezige, zieke rokweefsel ziet men donkere, concentrische ringen.*

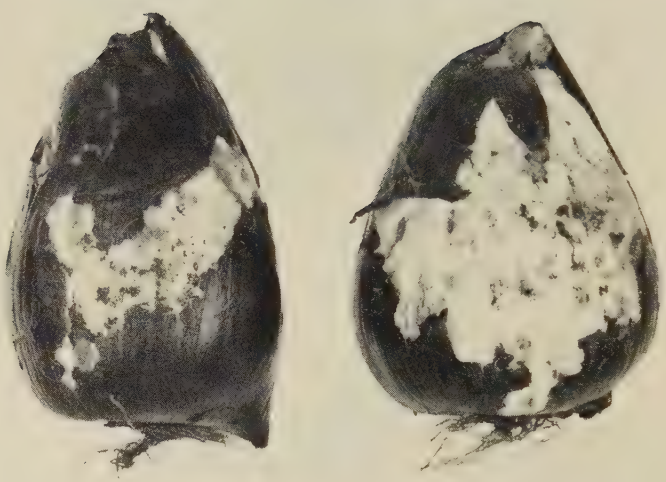


FIG. 6. Old corms of the *Colchicum* hybrid "Violet Queen" with partly removed diseased tunics.

Afgerijpte knollen van de Colchicum-*hybride „Violet Queen", waarvan het zieke rokgedeelte grotendeels heeft losgelaten.*

door *U. colchici* (fig. 2) zijn nl. slechts op twee plaatsen in Engeland geconstateerd, en wel in Kew (Massee, 1892, 1899 en 1915) en in Yorkshire (Mason & Grainger, 1937). De mogelijkheid van ondergronds optreden van deze roest was niet bekend.

Na een uitvoerige bespreking van de literatuurgegevens over *U. colchici* als veroorzaker van bovengrondse aantastingen, wordt de in Nederland waargenomen ondergrondse aantasting beschreven.

Bij de bovengenoemde *Colchicum*-hybriden, respectievelijk species, werden de teleutosori van *U. colchici* gevonden aan de binnenzijde en incidenteel ook aan de buitenzijde van de knolrok. Dit is uiteraard alleen vast te stellen na het rooien van de planten. Soms werden ook teleutosori gevonden op het stengelomvattende deel van de rok dat aansluit op de groene bladschede van het onderste blad. Hierdoor kan men soms in het veld even boven de grond aantastingsplekken waarnemen. Op de groene delen van de planten werden echter nooit sori gevonden; wel werden soms op de bladschijf van het onderste blad typische gele vlekjes waargenomen.

De aantasting is bijzonder opvallend als men de knollen vroeg rooit en het rokweefsel dus nog niet bruin verkleurd is (fig. 3). Later, als de rok bruin verkleurd en afgestorven is, is de roestaantasting de oorzaak dat het rokweefsel ter plaatse gaat scheuren en bij het rooien gedeeltelijk loslaat (fig. 5 en 6). Op het witte knolvlees blijft dan meestal als een bruinzwart poeder een aantal sporen achter (fig. 5 en 6). Een kenmerk van de aantasting in dit stadium is ook het optreden van vele donkerbruine, concentrische ringen in het afgestorven aangetaste rokweefsel (fig. 5B). Opvallend is, dat de rok vaak slechts aan één bepaalde zijde van de knol is aangetast. Meestal is dit een „zijkant" van de tweezijdig symmetrische knol (fig. 3A, 5, 6); soms worden de sori echter voornamelijk gevonden op het rokgedeelte aan de afgeplatte, verlengde zijde van de knol (fig. 3C).

In de discussie wordt er op gewezen, dat sinds 1956 een overeenkomstige ondergrondse roestaantasting bij *Crocus* wordt geconstateerd, veroorzaakt door *Uromyces croci* Pass. (Boerema, 1959). De mogelijkheid wordt overwogen, dat er bij deze twee in Nederland uitsluitend ondergronds optredende roesten sprake is van een adaptatie aan de cultuurmethode die bij deze planten op de kwekerijen reeds vele decennia wordt toegepast. Doordat de knollen op deze kwekerijen elk jaar weer uit de grond worden gehaald, is de bovengrondse levenscyclus van deze roesten zo goed als onmogelijk geworden, speciaal ook daar men hierbij steeds een ruime vruchtwisseling toepast. Met andere woorden deze roesten kunnen zich op de kwekerijen alleen handhaven als ze zich aanpassen aan de ondergrondse delen, wat dus blijkbaar nu is gebeurd. Deze hypothese houdt dus in dat hier mogelijk sprake is van erfelijk geadapteerde, fysiologische rassen van *U. colchici* en *U. croci*.

Verder worden in de discussie de gegevens over de levenscyclus van de bovengrondse aantasting door *U. colchici* vergeleken met de eigen waarnemingen over de biologie van de ondergrondse aantasting.

Bij de ondergrondse aantasting zullen de nieuwe infecties waarschijnlijk ontstaan door de basidiosporen van de in het voorjaar kiemende teleutosporen. Op deze wijze kan bij ziek plantmateriaal de vliezige rok van de binnen de oude knolrok gevormde, nieuwe knollen worden aangetast. Bij gezond plantmateriaal kan de infectie eventueel door in de grond aanwezige, oude, aangetaste rokfragmenten plaatsvinden.

Het feit dat de rok vaak slechts aan een „zijkant" van de knol is aangetast, bleek samen te hangen met de anatomie van *Colchicum*. De beide „zijkanten" en de afgeplatte, verlengde zijde van de nieuw gevormde knollen staan nl. in direct contact met de oude knolrok, terwijl de bolle zijde van de nieuwe knollen „beschermd" wordt door het knolvlees van het oude. Hierdoor is de kans van een infectie aan een zijkant van de nieuwe knollen het grootst.

Uitbreiding van de aantasting vindt waarschijnlijk plaats via de nerven (vgl. fig. 4), waardoor tenslotte ook teleutosori op het witte deel van de bladschede van het onderste blad kunnen ontstaan. Wat de oorzaak is dat er op de hoger gelegen, groene, dus chlorofyl bevattende delen geen teleutosori gevormd worden, moet nog nader worden onderzocht. Daar waar zich sori ontwikkelen, heeft ook een concentrische uitbreiding van het mycelium plaats, wat blijkt uit de meestal in concentrische ringen gerangschikte sori (fig. 3D) en de donkerbruine, concentrische ringen in het afgestorven rokweefsel (fig. 5B).

De publikatie wordt afgesloten met een aanvullende beschrijving van *Uromyces colchici*.

Acknowledgements

The author is very grateful to the Directions of the Herbaria at Kew, Leyden and Groningen for the excellent cooperation during this research.

My special thanks and appreciation are expressed to Mr. N. G. Santacroce for the contributions made by him on the underground appearance of *Uromyces colchici*, and for reviewing the English text.

From those who assisted in the preparation of this manuscript for publication, I wish to mention Mr. R. Surjotono especially, who carried out the preliminary investigations.

References

Anonymus, – 1956. Variëteitsnamen-register van Hyacinthen, Bol-Iris en andere diverse bijgoedgewassen. Kon. alg. Ver. Bloemb. Cult. Haarlem.

Boerema, G. H., – 1959. Uromyces croci Pass. bij Krokus-soorten. Versl. en Meded. Plantenz.-k. Dienst, Wageningen 133:130–135.

Boom, B. K. & J. D. Ruys, – 1950. Flora der gekweekte kruidachtige gewassen, Wageningen: 316.

Cooke, M. C., - 1906. Fungoid pests of cultivated plants: 277. (Reprinted from J. Roy. hort. Soc. 1902, 27: 400.)

Gäumann, E., – 1959. Die Rostpilze Mitteleuropas mit besonderer Berücksichtigung der Schweiz, Bern: 285–286.

Grove, W. B., – 1913. The British Rust Fungi (Uredinales). Cambridge.

Guyot, A. L., – 1951. Les Urédinées II. Encycl. mycolog. 15: 24–25; 46–55.

Guyot, A. L., – 1958. Les Urédinées. Corrections, Additions et Observations V in Uredineana V. Encycl. mycolog. 31: 713–760.

Horsfall, J. G. & A. E. Dimond, – 1959. Plant Pathology. New York, London: 307.

Hylander, M., I. Jorstad & J. A. Nannfeldt, – 1953. Enumerato Uredinearum Scandinavicarum. Opera Botanica 1: 1–102.

Mason, F. A. & I. Grainger, – 1937. A cataloque of Yorkshire Fungi. Yorkshire.

Massee, G., – 1892. Uromyces (Micruromyces) colchici, Mass. Grevillea 21: 6.

Massee, G., – 1899. A Text-book of Plant Diseases. London: 227.

Massee, G., – 1915. Diseases of cultivated plants and trees. London: 292.

Moore, W. C., – 1949. Diseases of bulbs. Bull. Min. Agric. London: 117: 57.

Moore, W. C., – 1959. British parasitic fungi. Cambridge: 402.

Ulbrich, E., – 1937. Die von der deutschen Himalaya-Expedition 1937 gesammelten Pilze. Notizbl. bot. Gart., Berlin-Dahlem 122, 14:150.

Unger, F., – 1833. Die Exantheme der Pflanzen. Wien: 186–187.

Wilson, M. & G. R. Bisby, – 1954. List of British Uredinales. Trans. Brit. mycol. Soc. 37:78.

T. Pl.-ziekten 67 (1961) 11–20

NEW DEVELOPMENTS IN THE SYSTEMIC COMBAT OF FUNGAL DISEASES OF PLANTS[1,2]

Met een samenvatting: Nieuwe ontwikkelingen in de bestrijding van schimmelziekten bij planten met systemisch werkzame middelen

BY

A. KAARS SIJPESTEIJN

Organisch Chemisch Instituut T.N.O., Utrecht, The Netherlands

INTRODUCTION

While systemic insecticides and herbicides are used in practice for several years already, the development of systemic fungicides seems to lag behind. None has as yet reached the stage of practical application.

Yet several compounds are known which, under experimental conditions, display a certain amount of systemic activity against fungi. In the ideal case such compounds should permeate the entire plant and combat the penetrating moulds or those already present. An interesting feature is the fact that the compounds displaying such activity are not necessarily fungicides or derivatives which can give rise to fungicides, for it is known that also certain compounds, which *in vitro* are inactive on moulds, are able to confer considerable protection to plants against fungal diseases.

It is not intended to give here a survey of all compounds showing systemic activity, but rather to consider how the compounds might act.

THE TEST

For an evaluation of systemic antifungal activity a test is required in which the three components: the plant, the fungus and the compound each play a role.

A much adopted test is the development of *Botrytis fabae* on broad bean, the well-known chocolate spot disease (cf. FAWCETT, SPENCER & WAIN, 1955). In the United States *Fusarium oxysporum* on tomato has often been used (cf. DAVIS & DIMOND, 1953). In our Research Unit for Internal Therapy of Plant Diseases cucumber scab caused by *Cladosporium cucumerinum* was introduced as a standard test object by VAN RAALTE (cf. VAN RAALTE et al., 1955). The economically important diseases, as for instance potato late blight, apple scab and Dutch elm disease, do not lend themselves to continuous experimental study. This is certainly unfortunate because we have no guarantee whatsoever that a compound which is active in one test, will also work against another disease.

Usually the compounds are applied to the plants one or more days prior to inoculation with the parasite, and in order to obtain a proper idea of the translocatability of the test compound, its place of application must be different from the spot where the disease is evaluated. We know already from the systemic in-

[1] After a lecture held at the IXth International Botanical Congress at Montreal in 1959.

[2] Accepted for publication 18 Nov., 1960.

secticides and herbicides that substances which are readily translocated upwards with the transpiration stream, do not necessarily also move downwards to any appreciable degree. The reverse is also found. Hence, root application as well as foliage application have to be studied in order to judge the effectiveness of a compound.

Systemic fungitoxic compounds

If we now turn to the first group of systemic compounds, namely the fungitoxic compounds, we may say that practice has taught us already that none of the present agricultural fungicides has appreciable systemic activity. Uptake and translocation by the plant of the known fungicides seem to be insufficient to give adequate systemic protection. Some compounds may be unable to penetrate, some may be adsorbed or metabolized by the plant, whereas other compounds are too toxic.

Yet various of the agricultural fungicides were shown to have some slight systemic activity against certain plant diseases as was shown by experiments of Grossmann (1957) with tetramethyl thiuramdisulfide against *Fusarium oxysporum* on tomato and of Volger (1959) with the same compound against diseases of seedlings of *Pinus silvestris*; Napier et al. (1957) showed systemic activity of "captan" against *Botrytis fabae* on broad bean and Rombouts & Kaars Sijpesteijn (1958) obtained systemic effects with pyridine-2-thiol-N-oxide against this latter disease and *Cladosporium cucumerinum* on cucumber. Also with the sulfate of 8-hydroxyquinoline limited results have often been described.

Antifungal antibiotics often show more promising effects than the synthetic compounds as systemic fungicides. Griseofulvin and actidione have received ample attention (cf. Brian, Wright, Stubbs & Way, 1951; Wallen & Millar, 1957). Rimocodin and pimaricin are able to combat the internal parasite *Ascochyta pisi* in pea seeds (Dekker, 1957).

We have to conclude, however, that uptake and translocation of fungicides on the whole are insufficient to effect satisfactory systemic protection. Thus we are bound to look for other methods in order to provide the plants with this systemic protection.

"*Masked fungicides*"

Since the first requirement for a systemic compound surely must be its translocatability, it is not surprising that one has tried to attach certain groups to fungicides with the aim of preparing better translocatable compounds. If this group masks the activity of the molecule, it should be split off again by the fungus or the plant at the desired site of action. Though this idea may seem rather bold, we may not forget that it is according to this principle that the systemic insecticides "schradan" and "demeton", which are nowadays applied in vast amounts, are doing their work.

In the Research Unit for Internal Theraphy for Plant Diseases it was originally attempted to follow this principle (van Raalte, Kaars Sijpesteijn, van der Kerk, Oort & Pluygers, 1955; van der Kerk, 1956). The investigation was based on the following observation of van Raalte (1952). The petiole of a potato leaf was placed on a plate seeded with *Penicillium italicum*. The length of this petiole cutting was 5 mm; on its top was placed a piece of filter

paper dipped in a solution of TMTD (1), TMTM (2) or sodium dimethyldithiocarbamate (3).

(1) $(CH_3)_2NC(=S)-S-S-C(=S)-N(CH_3)_2$ TMTD

(2) $(CH_3)_2NC(=S)-S-C(=S)-N(CH_3)_2$ TMTM

(3) $(CH_3)_2NC(=S)-SNa$ NaDDC

(4) $(CH_3)_2NC(=S)-SCH_2COOH$ carboxymethyl-DDC

After incubation a distinct zone of inhibition was seen around the petiole when TMTM was used; TMTD and NaDDC, however, produced no inhibition zone. This result led to the hypothesis that TMTM might be regarded as a dithiocarbamic acid derivative which is translocatable owing to the presence of a special group. Thus many compounds with the general formula $(CH_3)_2NCSSR$ were prepared. Their systemic activity was evaluated against cucumber scab. The recent dissertation of PLUIJGERS (1959) gives a summary of the compounds and the results obtained. The best compound which issued from this project, was the carboxymethyl derivative (4).

When cucumber seedlings are placed for two days with their roots in a solution of 500 p.p.m. of this compound and the foliage is subsequently sprayed with conidia of *Cladosporium cucumerinum*, the cotyledons and hypocotyls of the treated plant become far less attacked than those of the untreated plants.

How does carboxymethyl-DDC act? The compound itself is only very slightly fungitoxic *in vitro*, but it was intended to be decomposed in the tissues and to give rise there to the fungitoxic dithiocarbamate. It may, however, be doubted, if in fact carboxymethyl-DDC acts according to this principle, because sap expressed from cucumber plants treated with this compound does not show fungicidal activity on *Cladosporium cucumerinum in vitro*. Neither was there activity on *Glomerella cingulata*, a mould which is much more sensitive to NaDDC than *Cladosporium cucumerinum*.

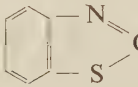
The following observation may offer another possible explanation for the systemic activity of carboxymethyl-DDC. When VAN RAALTE studied the merits of this compound against diseases of tomato plants, he was struck by a curious effect on these plants, for they showed the same picture as plants treated with 2,4-dichloro phenoxyacetic acid (2,4-D) namely a growth-regulator effect. Further studies revealed that carboxymethyl-DDC must indeed be regarded as a true plant growth regulator (VAN DER KERK, VAN RAALTE, KAARS SIJPESTEIJN & VAN DER VEEN, 1955).

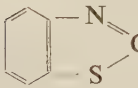
The fact that growth regulators may give systemic protection was at that time already a familiar phenomenon since DAVIS & DIMOND (1953) as well as other investigators had found that various plant growth regulators could induce sys-

temic protection to various diseases. Like carboxymethyl-DDC they are not fungitoxic *in vitro*.

Seen in this light it seems acceptable that the mode of action of carboxymethyl-DDC is the same as that, which is assumed for the other growth regulators, namely not a direct action on the parasite, but an interference with plant metabolism which leads to increased resistance of the plants to the parasite.

The case of carboxymethyl-DDC even shows a striking similarity with that of S-carboxymethyl-2-mercaptobenzothiazole (5) (cf. DAVIS & DIMOND, 1953). Both are derived from a fungicide (3 and 6 respectively) by attachment of a carboxymethyl group. Both are growth regulators and bring about systemic protection. This systemic protection must be ascribed rather to an increase of host resistance than to a decomposition to the fungitoxic principle.

(5)  $CSCH_2COOH$ S-carboxymethyl-2-mercaptobenzothiazole

(6)  CSH 2-mercaptobenzothiazole

In a similar way certain groups were added to the molecule of the antibiotic "actidione". HAMILTON, SZKOLNIK & SONDHEIMER (1956) found that the oxime, the semicarbazone and the acetate had appreciable systemic activity against cherry leaf spot (*Coccomyces hiemalis*), whereas "actidione" itself was inactive. It is supposed that the three active compounds are translocated as such to the new leaves where they are broken down again to the fungicidal "actidione". The same derivatives were found more active than "actidione" itself in the systemic control of wheat rust (WALLEN, 1958).

Up till now we have considered the improvement of translocatability by attachment of certain groups to the molecule; translocatability of fungicides can, however, also be influenced by formation of certain salts. Thus BAKKEREN (1958) reported that the cetylpyridinium salt of dimethyldithiocarbamic acid has a curative effect on apple scab. It can still be applied 3 days after infection, because it penetrates into the plant and kills the fungus there.

If we consider the results obtained up till now, we will have to admit that the improvement of translocatability of fungicides by attachment of labile groups to the molecule has not yielded a striking success in the field of systemic fungicides. This may be due to a large extent to our almost complete lack of knowledge of the relation between structure and uptake of organic compounds into the plants as well as their distribution and stability in the tissues.

Translocation studies

A first attempt to study the relation between structure and translocatability was made by CROWDY, ELIAS & RUDD JONES (1958) for a group of sulfonamides. In a typical experiment broad beans were placed for 13 days with their roots in a 0.01 % solution (= 100 p.p.m.) of these compounds. Subsequently the concentration of the compounds in the leaves was assessed. The following concentrations were found per fresh weight of the leaves: sulfanilamide 279 p.p.m., sulfa-

guanidine 75 p.p.m., sulfadiazine 218 p.p.m., sulfacetamide 228 p.p.m., sulfathiazole 44 p.p.m. and N^4-acetyl sulfanilamide 383 p.p.m. This result illustrates the differences in uptake and translocatability. It also shows that the concentration inside the plant may become higher than that of the solution in which the roots were placed. Of course, experiments of this kind do not give any information about the micro-distribution of the compounds within the leaves.

Ross & Ludwig (1957) made a quantitative study of the translocation of a series of ethylenethiourea derivatives in tomato plants. N-Octylethylenethiourea accumulated readily in the root system, whereas N-amyl- and N-ethylethylenethiourea are much more mobile and move upwards.

These two examples may stress the importance of further quantitative work on translocatability of organic compounds.

Stability in the plant

The systemic effect of a compound is not only determined by translocatability; the stability in the plant certainly is another factor of importance. As soon as a compound enters the plant cells, it becomes exposed to their full metabolic potential. Some compounds appear to be stable under these conditions, other compounds will be broken down. Special attention will be drawn here to the fact that foreign compounds may also become involved in synthetic reactions in the plant.

Better translocatable and less phyto- and fungitoxic substances can arise in this way. Thus sulfanilamide is transformed to a large extent into the less fungitoxic N^4-acetyl derivative; an equilibrium seems to exist since the latter compound in its turn gives rise to the former when taken up by the plant (Crowdy & Rudd Jones, 1958). Another example is the transformation of the herbicide aminotriazole into a glucoside (Rogers, 1957) or into an alanine-aminotriazole derivative (Massini, 1959). Phenolic compounds are subject to β-glycosidation in the plant as was shown by several investigators (cf. Hutchinson, Roy & Towers, 1958 and Winter & Schönbeck, 1959). That similar reactions occur following the uptake of the fungicide sodium dimethyldithiocarbamate by various plants is suggested by the work of Dekhuijzen (in the press).

This kind of biochemical transformation of fungicides in the plant can be of advantage for the systemic combat of fungi if the new product is translocatable and if it is sufficiently fungitoxic itself or is broken down again by the moulds to give rise to the original fungicide.

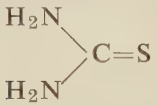
Systemic compounds which are not fungitoxic in vitro

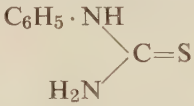
After having dealt with the systemic activity of fungicides and some of their derivatives we will now turn to that most interesting group of compounds which do show systemic protection, although they are not fungitoxic *in vitro*.

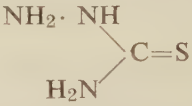
An almost classic example is the observation of Davis & Dimond (1953) that plant growth regulators when applied to the roots protect tomatoes against *Fusarium oxysporum*. Their protective action is ascribed to interference with plant metabolism in such a way that resistance of the plant to the parasite results. There are many more examples of the action of growth regulators on plant diseases, but we will later come back to these compounds.

Thioureas. An interesting group of compounds seems to be thiourea with some derivatives. It was found by VAN ANDEL & PLUIJGERS (1957, unpublished results) that thiourea (7) as well as phenylthiourea (8) rendered cucumber plants resistant to attack by *Cladosporium cucumerinum* after application to roots or foliage. They are in fact much more active compounds than carboxymethyl-DDC, since protection is almost complete. At the same time KUC, WILLIAMS & SHAY (1957) published a short note in which they claimed that phenylthiourea showed systemic activity against apple scab.

FUCHS & BAUERMEISTER (1958) could suppress the development of wheat rust by treatment of the leaves with a derivative of thiourea namely thiosemicarbazide (9). Its mode of action may be the same as that of thiourea.


(7)


(8)


(9)

How do these compounds act? As much as 500 p.p.m. is required for inhibition *in vitro*. It is not impossible that the concentration inside the plants reached this level. Yet the sap expressed from the leaves of cucumber plants treated with phenylthiourea shows no fungitoxic activity. Hence it seems rather that the compounds act by interfering with plant metabolism. Thioureas, after all, are known to react strongly with certain plant enzymes, as for instance the polyphenoloxidase.

Sulfonamides. As we have seen the work of CROWDY, ELIAS & RUDD JONES (1958) has shown that sulfonamides are easily translocated. They found that these compounds also bring about very effective systemic protection to rust of beans and wheat and to mildew of wheat and oats. It is left undecided whether this effect is due to direct action on the fungi or to interference with host metabolism.

Streptomycin. Another example is the control of *Phytophthora* in potato by streptomycin (MÜLLER, MACKAY & FRIEND, 1954). The action of streptomycin which is a bactericide but not a fungicide is ascribed by VÖRÖS, KIRALY & FARKAS (1957) to a stimulating action of streptomycin on the polyphenoloxidase activity of the plant.

Amino acids. Thioureas, sulfonamides and streptomycin are foreign compounds which alter the resistance of the plants by interference with plant metabolism. It may now be asked in how far resistance of the plant can be also altered by addition of plant metabolites. Indole acetic acid was already found active. KUC and coworkers (1957, 1959) studied the action of amino acids on apple scab and found phenylalanine and alanine to be very active. VAN ANDEL (1958) reported systemic activity of the amino acids serine and threonine against cucumber scab. It seems, however, most remarkable that KUC and coworkers as well as VAN ANDEL found that only the D-isomers are active, whereas the natural L-isomers are practically ineffective. Also the unnatural amino acid L-threo-β-phenylserine is very active against cucumber scab. The mechanism of their activity is still unknown, but it is supposed to be an interference with plant metabolism (OORT & VAN ANDEL, 1960).

Sugars. The addition of sugars also can seriously affect resistance. I will mention here only an example of YARWOOD (1934) who found that the addition of saccharose to clover leaflets reduced their susceptibility to *Stemphylium sarciniforme*. At the same time it increased their susceptibility to powdery mildew. The sugar level in fact appears to determine the degree of susceptibility of many plants towards various diseases. This concept was discussed by HORSFALL & DIMOND (1957) who distinguish "low sugar diseases" and "high sugar diseases". Any chemical that alters the sugar level in the plant, can thus be expected to alter susceptibility to these diseases in one way or another. As examples for low sugar diseases *Alternaria* on tomato and Dutch elm disease are mentioned; diseases caused by mildews and rusts and also *Botrytis fabae* on broad beans would belong to the high sugar diseases.

Growth regulators. On this basis HORSFALL & DIMOND (1957) have forwarded an elegant theory to explain the effect of growth regulators on the resistance to plant diseases. They point to the fact that these compounds after a slight initial increase, cause a fast decrease of the sugar level of foliage so that the level eventually falls below that of untreated plants. Thus growth regulators would induce plants to become more resistant to a high sugar disease and this is indeed so in many cases. Alternatively, treatment with growth regulators would induce plants to become more susceptible to low sugar diseases because the growth regulators lower the sugar level. This extremely attractive theory gives an explanation for many results obtained after treatment with growth regulators.

It is, moreover, intriguing that growth regulators as well as certain circumstances which affect the sugar level in the plant are also known to influence the development of certain virus diseases and bacterial diseases. Hence we may even ask in how far the sugar level can also determine susceptibility to these diseases.

In how far does the systemic activity of the growth regulator carboxymethyl-DDC fit into this picture? Does it act by lowering the sugar level in the cucumber plant and is cucumber scab a high sugar disease?

Evidence for the latter suggestion was readily obtained (KASLANDER & KAARS SIJPESTEIJN). When cucumber seedlings were placed in a glucose solution prior to spraying with the parasite the sugar level was high and disease became more severe than in the control plants. When alternatively plants were kept at low light intensity before and during incubation, the sugar level was low and disease became less severe. If, however, glucose was added simultaneously, it apparently counteracted the effect of darkness, the glucose level was high and disease was more severe again. These observations pointed definitely to a high sugar disease.

Our expectation was that treatment with the growth regulator carboxymethyl-DDC would lower the sugar level in cucumber seedlings. This frequently was found to be the case. If, moreover, carboxymethyl-DDC was given together with glucose, the suger level in the plant was high and disease severe. Thus we are inclined to believe that the protective effect of carboxymethyl-DDC is to a certain extent related to a decrease in sugar level in the plant. Yet other factors probably also play a role. It seems, moreover, an oversimplification to assume that the sugar level should influence susceptibility directly, because other factors, which are influenced by the sugar level, as for instance the osmotic value of the cell sap, might also be determinative for the degree of susceptibility.

Recently CORDEN & DIMOND (1959) offered a very different explanation for the systemic control of *Fusarium* in tomato by growth regulators. A correlation was found here between systemic control and inhibition of root elongation. The systemic control is thought to be due to the different state of the petic substances in the shorter roots (EDDINGTON & DIMOND, 1957).

The theory that growth regulators act by changing the sugar level might also offer an explanation for the fact that not only growth regulators themselves, but also closely related compounds without growth-regulating properties can have systemic effects against plant diseases.

Thus in England WAIN and coworkers prepared an impressive number of growth regulators, and related substances without growth-regulating effect. Many of these evoke some systemic protection against chocolate spot disease (cf. FAWCETT, SPENCER & WAIN, 1957). It would be of interest to know if the growth regulators which give systemic protection influence the sugar level in the same way as those analogues which are no growth regulators but which do give systemic protection.

It may be asked how far reaching the concept of HORSFALL & DIMOND will prove to be. Not only does it give an approach to the understanding of the effect of growth regulators on fungal diseases, it shows also quite generally how important the metabolic state of the host plant may be for susceptibility. This does not only apply to carbohydrate metabolism, also nitrogen metabolism of the host plays a role in determining the susceptibility of the plant. Carbohydrate metabolism and nitrogen metabolism are, moreover, closely interconnected.

The role which the metabolic state of the plant plays in susceptibility to disease is a field which, at its biochemical level, is only at the start of its exploration. Here we also touch the interesting field of natural resistance. For natural resistance only rarely depends on anatomical properties of the plant or on the presence of fungitoxic substances; it probably is more often determined by biochemical conditions. Knowledge of essential biochemical differences between resistant and susceptible races of the same plant species may therefore well have a stimulating effect on the development of systemic compounds which act by increasing the resistance of the plant.

In conclusion it may be stated that the development of systemic compounds which act by increasing the resistance of the host plant is as important as the development of systemic fungicides.

SUMMARY

A survey is given of some recent developments in the field of the systemic combat of fungus diseases of plants. The stage of practical application has not yet been reached.

Several examples are given of compounds which act by direct fungitoxic activity and of compounds which act by increasing host resistance.

SAMENVATTING

Er wordt een overzicht gegeven van enkele nieuwe ontwikkelingen op het gebied van de bestrijding van schimmelziekten bij planten met systemisch werkzame middelen. Het stadium van praktische toepassing van dergelijke middelen is nog niet bereikt.

Verschillende voorbeelden worden gegeven van stoffen die hun activiteit ontlenen aan directe fungitoxische werking en ook van stoffen die werkzaam zijn doordat zij de resistentie van de gastheer verhogen.

References

ANDEL, O. M. VAN, – 1958. Investigations on plant chemotherapy II. Influence of amino acids on the relation plant - pathogen. T. Pl.-ziekten 64: 307-327.

BAKKEREN, M., – 1958. Enige nieuwe ontwikkelingen op fungiciden gebied. T. Pl.-ziekten 64: 121.

BRIAN, P. W., J. M. WRIGHT, J. STUBBS & A. M. WAY, – 1951. Uptake of antibiotic metabolites of soil micro-organisms by plants. Nature 167: 347–349.

CORDEN, M. E. & A. E. DIMOND, – 1959. The effect of growth-regulating substances on disease resistance and plant growth. Phytopathology 49: 68–72.

CROWDY, S. H., R. S. ELIAS & D. RUDD JONES, – 1958. The control of certain plant diseases with sulphonamides. Ann. appl. Biol. 46: 149–158.

CROWDY, S. H. & D. RUDD JONES, – 1958. The translocation of sulphonamides in higher plants III. Acetylation and deacetylation of sulphanilamide in broad beans and wheat. J. exp. Bot. 9: 220–228.

DAVIS, D. & A. E. DIMOND, – 1953. Inducing disease resistance with plant growth-regulators. Phytopathology 43: 137–140.

DEKHUIJZEN, H. M., – 1961. On the transformation in plants of sodium dimethyldithiocarbamate into other fungitoxic compounds. Nature: In the press.

DEKKER, J., – 1957. Inwendige ontsmetting van door Ascochyta pisi aangetaste erwtezaden met de antibiotica rimocidine en pimaricine, benevens enkele aspecten van het parasitisme van deze schimmel. T. Pl.-ziekten 63: 65–144.

DIMOND, A. E. & D. DAVIS, – 1953. The chemotherapeutic activity of benzothiazole and related compounds for Fusarium wilt of tomato. Phytopathology 43: 43–44.

EDGINGTON, L. V. & A. E. DIMOND, – 1959. Correlation between the wilt resistance of plants and the nature of their pectic substances. Phytopathology 49: 538.

FAWCETT, C. H., D. M. SPENCER & R. L. WAIN, – 1955. Investigations on fungicides I. Fungicidal and systemic fungicidal activity in certain aryloxyalkanecarboxylic acids. Ann. appl. Biol. 43: 553–568.

FAWCETT, C. H., D. M. SPENCER & R. L. WAIN, – 1957. Investigations on fungicides II. Aryloxy- and arylthio-alkanecarboxylic acids and their activity as fungicides and systemic fungicides. Ann. appl. Biol. 45: 158–176.

FUCHS, W. H. & R. BAUERMEISTER, – 1958. Ueber die Wirkung von Thiosemicarbazid auf die Entwicklung von Puccinia graminis tritici auf Weizen. Naturwiss. 45: 343–344.

GROSSMANN, F., – 1957. Untersuchungen über die innentherapeutische Wirkung organischer Fungizide I. Thiocarbamate und Thiurame. Z. Pfl.-Krankh. Pfl.-Sch. 64: 718–728.

HAMILTON, J. M., M. SZKOLNIK & E. SONDHEIMER, – 1956. Systemic control of cherry leafspot fungus by foliar sprays of actidione derivatives. Science 123: 1175–1176.

HORSFALL, J. G. & A. E. DIMOND, – 1957. Interactions of tissue sugar, growth substances and disease susceptibility. Z. Pfl.-Krankh. Pfl.-Sch. 64: 415–421.

HUTCHINSON, A., C. ROY & G. H. N. TOWERS, – 1958. Synthesis of phlorin and other phenolic glucosides by plant tissues. Nature 181: 841.

KASLANDER, J. & A. KAARS SIJPESTEIJN, – To be published.

KERK, G. J. M. VAN DER, – 1956. The present state of fungicide research. Meded. Landbouwhogesch. Gent 21: 305–339.

KERK, G. J. M. VAN DER, M. H. VAN RAALTE, A. KAARS SIJPESTEIJN & R. VAN DER VEEN, – 1955. A new type of plant growth-regulating substances. Nature 176: 308–310.

KUC, J., E. BARNES, A. DAFTSIOS & E. B. WILLIAMS, – 1959. The effect of amino acids on susceptibility of apple varieties to scab. Phytopathology 49: 313–315.

KUC, J., E. B. WILLIAMS & J. R. SHAY, – 1957. Increase of resistance to apple scab following injection of host with phenylthiourea and D-phenylalanine. Phytopathology 47: 21.

MASSINI, P., – 1959. Synthesis of 3-amino-1, 2, 4-triazolyl alanine from 2-amino-1, 2, 4-triazole in plants. Biochim. Biophys. Acta 36: 548–549.

MÜLLER, K. O., J. H. E. MACKAY & J. N. FRIEND, – 1954. Effect of streptomycin on the host-parasite relationship of a fungal phytopathogen. Nature 174: 878–879.

Napier, E. J., A. Rhodes, D. I. Turner, J. Tootill & A. Dunn, – 1957. Systemic control of captan against Botrytis fabae (chocolate spot of broad bean). J. Sci. Food. Agr. 8: 467–473.
Oort, A. J. P. & O. M. van Andel, – 1960. Aspects of chemotherapy. Meded. Landbouwhogesch. Gent 25. In the press.
Pluijgers, C. W., – 1959. Direct and systemic antifungal action of dithiocarbamic acid derivatives. Dissertation, Utrecht.
Raalte, M. H. van, – 1952. A test for the translocation of fungicides through plant tissues. C. R. III. Congr. int. Phytopharm., Paris: 76–78.
Raalte, M. H. van, A. Kaars Sijpesteijn, G. J. M. van der Kerk, A. J. P. Oort & C. W. Pluijgers, – 1955. Investigations on plant chemotherapy. Meded. Landbouwhogesch. Gent 20: 543–555.
Rogers, B. J., – 1957. ATA. The action of 3-amino-1,2,4-triazole in plants. The Hormolog, February: 10–12.
Rombouts, J. E. & A. Kaars Sijpesteijn, – 1958. The chemotherapeutic effect of pyridine-2-thiol-N-oxide and some of its derivatives on certain plant diseases. Ann. appl. Biol. 46: 30–36.
Ross, R. G. & R. A. Ludwig, – 1957. Fungitoxicity and phytotoxicity in a homologous series of N-n-alkylethylene thioureas. Can. J. Bot. 35: 65–95.
Volger, C., – 1959. Ueber die Möglichkeit einer systemischen Wirkung von TMTD-Präparaten. Meded. Landbouwhogesch. Gent 24: 837–849.
Vörös, J., Z. Király & G. L. Farkas, – 1957. Role of polyphenolase in streptomycin-induced resistance to Phytophthora in potato. Science 126: 1178.
Wallen, V. R., – 1958. Control of stem rust of wheat with antibiotics II. Systemic activity and effectiveness of derivatives of cycloheximide. Pl. Dis. Rep. 42: 363–366.
Wallen, V. R. & R. L. Millar, – 1957. The systemic activity of cycloheximide in wheat seedlings. Phytopathology 47: 291–294.
Winter, A. G. & F. Schönbeck, – 1959. Untersuchungen über die Aufnahme organischer Substanzen durch die Wurzeln höherer Pflanzen I. Phenolische Verbindungen. Naturwiss. 18: 536–537.
Yarwood, C. E., – 1934. The comparative behavior of four clover leaf parasites on excised leaves. Phytopathology 24: 797–806.

T. Pl.-ziekten 67 (1961): 21–24

THE TOXICITY OF SOME FUNGICIDES TO CONIDIA OF *GLOEOSPORIUM FRUCTIGENUM* F. *CHROMOGENUM*[1]

Met een samenvatting: De toxiciteit van enkele fungiciden voor de conidiën van Gloeosporium fructigenum *f.* chromogenum

BY

G. J. M. A. GORTER

Division of Plant Pathology, Department of Agriculture, Pretoria, South Africa

INTRODUCTION

Anthracnose of olives is a wide-spread plant disease caused by various species of the *Glomerella* group of fungi (GORTER, 1959). In South Africa it is caused by *Gloeosporium fructigenum* Berk. f. *chromogenum* Gorter (GORTER, 1956, 1959). This fungus can be successfully combated by spraying with home-made Bordeaux mixture (GORTER, 1960).

For treating fruit tree diseases in this country home-made Bordeaux mixture is now rapidly being replaced by factory-made fixed copper sprays and organic fungicides. It was therefore considered desirable to test these fungicides against olive anthracnose.

Comparative field tests would require very large and uniform olive groves. In South Africa there are no such plantings where anthracnose is troublesome. This is because the disease is invariably found only near the coast and here the soil usually slopes and the fertility varies. The fungicides had therefore to be evaluated in spore germination tests only.

METHODS AND MATERIALS

A modification of the slide-germination technique recommended by the American Phytopathological Society (1943, 1947) was used.

Each fungicide was mixed with distilled water and diluted. The dilutions contained respectively 10,000, 1000, 100, 10 and 1 part per million of the fungicide. According to need further dilutions were interpolated to obtain germination percentages between 0 and 100.

Three 3 × 1 inch glass slides, each with two concavities, were cemented with Canada balsam to a 3 × 3 inch glass plate, giving six depressions per plate. This is a modification of the method of HARTSUIJKER (1940). A solid glass rod was used to put one drop of the fungicidal solutions into each of the cavities. The plates were then dried in an oven at 50 °C. Distilled water was used as a control.

A spore suspension was made by shaking distilled water with 2–3 week-old cultures of the test fungus for one minute, and filtering the resulting spore suspension through glass wool to remove mycelium fragments. It was diluted until no more than 10 spores could be found in each micrometer frame of a

[1] Accepted for publication 1 Nov., 1960.

net-oculair at a magnification of 160 ×. The spore suspension was then mixed with an equal volume of a stimulant consisting of 0.1 per cent dextrose and the mineral salts magnesium sulphate, ammonium chloride and potassium dihydrogen phosphate in 2.0 millimol concentrations. Although opinions differ on the advisability of adding a stimulant (TOMKINS, 1932; WILCOXON & MCCALLAN, 1934; HARTSUIJKER, 1940; HORSFALL et al., 1940) there was no choice in this case, because the spores of the olive antracnose fungus germinated very poorly in distilled water alone. This was similar to what LINN (1945) found for *Glomerella cingulata*.

With a graduated pipette 0.08 cc of the spore suspension was put in each concavity of the prepared plates. The cultures were then incubated at 26 °C – about the optimum temperature for growth of the fungus – for 8 hours and then examined for germination of spores. It is usual to incubate such cultures at least 24 hours before examination, but with *Gloeosporium fructigenum* f. *chromogenum* secondary spores are formed within 24 hours, so a shorter period of incubation is necessary. According to WELLMAN & MCCALLAN (1942), the germination results are just as accurate after 6 or 12 hours as after 24 or 48 hours, provided the temperature is kept constant; the importance of the temperature increases as the observation time decreases.

Fifty spores in each culture drop were examined and the number that had germinated was noted. As four replicates of each fungicide concentration had been prepared concurrently, 200 spores were examined for each dilution. This number is adequate for the degree of accuracy aimed at in these experiments (BLUMER & KUNDERT, 1950).

The following copper fungicides were compared: Bordeaux mixture ("Capex" brand), copper oxychloride ("Cupravit") and cuprous oxide ("Perenox"). In addition five organic fungicides were tested viz. ferbam ("Fermate") maneb ("Manzate"), zineb ("Parzate"), captan ("Flit 406") and dichlone ("Phygon XL") of which the first three are dithiocarbamates.

RESULTS AND CONCLUSIONS

For each fungicide the germination figures for the various dilutions were multiplied by a factor $\frac{100}{x}$ in which x was the mean percentage of germinated spores in the controls. The percentage of non-germinated spores obtained in the final dilution series for each fungicide was used for the expression of the response to these fungicides in toxicity curves. These curves were drawn on ordinary graph paper after converting the percentages of non-germinated spores into probits – the probability units devised by BLISS (1935) – and plotting their values against the logarithms of the corresponding dilutions. From these dosage-response curves the E.D. 50 as well as the E.D. 95 were determined for each fungicide. Their values can be found in table 1.

The figures in the table show that there are slight but noticeable differences in the effects of the three copper preparations. Copper oxide was the best and copper oxychloride the worst. Of the three dithiocarbamates ferbam was the most effective and zineb the least. Zineb was even less toxic to the spores than was any of the copper fungicides. This is an interesting result as a similar

TABLE 1. E. D. Values of eight different fungicides for preventing germination of *Gloeosporium fructigenum* f. *chromogenum* conidia.

E.D.-waarden van acht verschillende fungiciden ter voorkoming van ontkieming van conidiën van Gloeosporium fructigenum *f.* chromogenum.

Fungicide	E.D. 50 Parts per million *Delen per miljoen*	E.D. 95 Parts per million *Delen per miljoen*
Bordeaux mixture/*Bordeauxse pap* . .	331	2,750
Copper-oxychloride/*Koper-oxychloride*	502	6,760
Cuprous oxide/*Cupro-oxide*	191	1,410
Ferbam	0.22	0.49
Maneb	468	832
Zineb	955	4,680
Captan	32.4	123
Dichlone	66.1	302

relationship between zineb and Bordeaux mixture was noted in spray trials for the control of olive anthracnose in Italy (GRANITI, 1956). The results obtained with maneb in the germination tests were more or less of the same order as those of the copper fungicides. The remaining organic fungicides, captan and dichlone were both more effective than the copper sprays but not as effective as ferbam. Thus the only preparations worthy of further testing under field conditions are ferbam, captan and dichlone.

SUMMARY

The comparative efficacy of a number of fungicides was tested on the conidia of *Gloeosporium fructigenum* Berk. f. *chromogenum* Gorter, the cause of olive anthracnose in South Africa. The tests were based on the slide-germination technique recommended by the American Phytopathological Society and modified to suit local conditions. It was necessary to use a stimulant to encourage germination.

The tests showed that cuprous oxide was slightly better than Bordeaux mixture but that copper oxychloride and zineb were not as good. Captan and dichlone were appreciably more effective than Bordeaux mixture. Ferbam was the most effective of all the fungicides tested.

SAMENVATTING

Wegens de moeilijkheden welke bij praktijkproeven voor het gelijktijdig vergelijken van een aantal fungicide spuitmiddelen in plaatselijke boomgaarden van olijven ondervonden worden, werd de doeltreffendheid van enkele nieuwere koper- en organische schimmeldodende preparaten voor het bestrijden van *Gloeosporium fructigenum* Berk. f *chromogenum* Gorter, de oorzaak van de olijf-anthracnose in Zuid-Afrika, in sporekiemingsproeven bestudeerd.

De gevolgde techniek was met enkele wijzigingen gebaseerd op een door de Amerikaanse Fytopathologische Vereniging aanbevolen methode. In plaats van gewone objectglazen werden echter vierkante glasplaatjes gebruikt, waarop naast elkaar drie objectglaasjes, elk van twee uithollingen voorzien, met

canadabalsem vastgekleefd waren. Om van een goede en gelijkmatige ontkieming van de sporen verzekerd te zijn, was het noodzakelijk een stimulans toe te voegen, waarvoor een oplossing werd gekozen die voor *Glomerella cingulata* doeltreffend was bevonden. Waarnemingen over de ontkieming werden na een tijdsduur van acht uren verricht om te voorkomen dat de vorming van secundaire sporen het kiemingsbeeld vertroebelden.

Drie koperpreparaten en vijf organische fungiciden werden aan de toets onderworpen. De verkregen resultaten zijn af te lezen uit tabel 1. Hoewel het verschil in reactie tussen de drie koperverbindingen gering is, werd er toch een duidelijke aanwijzing gevonden, dat cupro-oxide iets sterker en koper-oxychloride iets zwakker op de sporen inwerkt dan Bordeauxse pap. Bij de dithiocarbamaten deden zich echter opvallender verschillen voor. Zo bleek ferbam een uiterst effectief middel om de sporekieming bij *G. fructigenum* f. *chromogenum* te onderdrukken en is het zelfs het meest toxische van alle onderzochte preparaten. De maneb- en zineb-preparaten waren veel minder effectief; zineb reageerde zelfs zwakker dan Bordeauxse pap. Captan en dichlone daarentegen onderdrukten de kieming weer veel sterker dan Bordeauxse pap, maar niet zo effectief als ferbam.

Van de onderzochte preparaten komen dus alleen ferbam, captan en dichlone in aanmerking voor verdere praktijkproeven om de anthracnose van olijven te bestrijden.

References

American Phytopathological Society, – 1943. The slide-germination method of evaluating protectant fungicides. Phytopathology 33: 627–632.

American Phytopathological Society, – 1947. Test tube dilution technique for use with the slide-germination method of evaluating protectant fungicides. Phytopathology 37: 354–356.

Bliss, C. I., – 1935. The calculation of the dosagemortality curve. Ann. appl. Biol. 22: 134–167.

Blumer, S. & J. Kundert, – 1950. Methoden der biologischen Laborprüfung von Kupferpräparaten. Phytopath. Z. 17: 161–199.

Gorter, G. J. M. A., – 1956. Anthracnose fungi of olives. Nature 178: 1129–1130.

Gorter, G. J. M. A., – 1959. Onderzoekingen over Gloeosporium fructigenum Berk. f. chromogenum Gorter form. nov., de oorzaak van de anthracnoseziekte van olijven in Zuid-Afrika. Unpublished D.Sc. (Agric.) Thesis, University of Pretoria.

Gorter, G. J. M. A., – 1960. Control of anthracnose in olives. Fmg. S. Afr. 36(3): 18–19, 22, 24.

Graniti, A., – 1956. Prove di lotta contro la „lebbra" della olive in Sicilia con calendari misti, utili anche contro la „mosca". Riv. Olearia (7–8): 3–16.

Hartsuijker, K., – 1940. Het wetenschappelijk onderzoek van fungiciden. Thesis, University of Amsterdam.

Horsfall, J. G., J. W. Heuberger, E. G. Sharwelle & J. M. Hamilton, 1940. A design for laboratory assay of fungicides. Phytopathology 30: 545–563.

Linn, C. K., – 1945. Nutrient requirements in the germination of conidia of Glomerella cingulata. Am. J. Bot. 32: 296–298.

Tomkins, R. G., – 1932. Measuring germination. Trans. Brit. Myc. Soc. 17: 147–149.

Wellman, R. H. & S. E. A. McCallan – 1942. An analysis of factors causing variation in spore germination tests of fungicides IV. Time and temperature. Contr. Boyce Thompson Inst. 12: 431–449.

Wilcoxon, F. & S. E. A. McCallan, – 1934. The stimulation of fungus spore germination by aqueous plant extracts. Phytopathology 24:20.

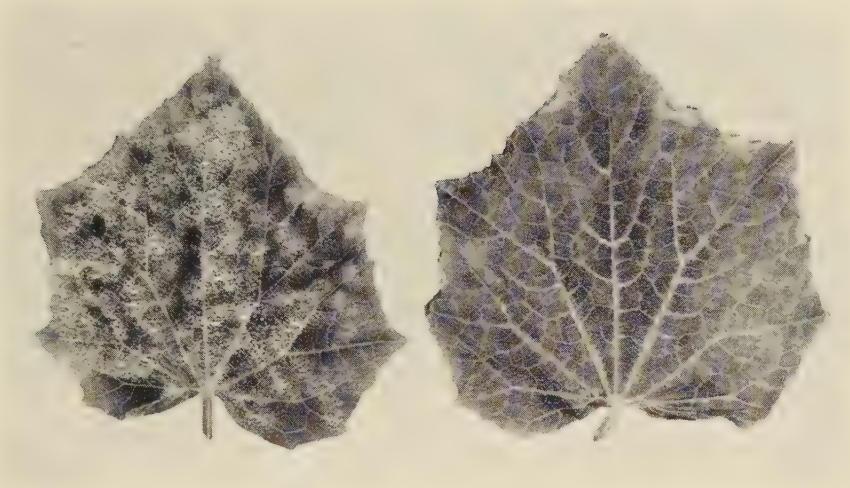


FIG. 1. Powdery mildew on cucumber leaves.
Right: Leaf from plant sprayed with procaine HCl 1000 p.p.m. on first and second day after inoculation.
Left: Leaf from control plant, sprayed with water.

Echte meeldauw op komkommerbladeren.
Rechts: Blad van plant, op de eerste en tweede dag na de inoculatie bespoten met procaïne-HCl (1000 d.p.m.).
Links: Blad van controle-plant, bespoten met water.

T. Pl.-ziekten 67 (1961): 25–27

SYSTEMIC ACTIVITY OF PROCAINE HYDROCHLORIDE ON POWDERY MILDEW[1]

Met een samenvatting: Systemische werking van procaïne-hydrochloride tegen echte meeldauw

BY

J. DEKKER

Werkgroep Interne Therapie bij Planten, T.N.O.,
Laboratorium voor Fytopathologie, Wageningen

Investigating control of plant diseases with antibiotics at the University of California, DR. P. A. ARK (personal communication) observed systemic activity of a commercial penicillin preparation (procaine penicillin G) against powdery mildew on cucumbers after administering this antibiotic to the roots. Follow up of this observation at our laboratory indicated that not penicillin G but the procaine part of the molecule was responsible for the chemotherapeutic effect. The following is a preliminary report on the activity of procaine hydrochloride against several powdery mildews of plants.

Cucumber plants, variety "Lange gele tros", were grown on HOAGLAND nutrient solution. After development of two leaves, the plants were inoculated with a conidial suspension of *Erysiphe cichoracearum* DC, and procaine HCl was added to the nutrient solution. Transfer of the plants to a procaine free medium took place after 4 days and assessment for mildew after 14 days, using a scale ranging from 0, no mildew, to 5, leaf covered with mildew.

TABLE 1. Powdery mildew on cucumber plants, after addition of procaine HCl to the nutrient solution for 4 days. Average of 6 plants each with 2 leaves.
Ontwikkeling van meeldauw op komkommerplanten, die gedurende 4 dagen na de inoculatie op een voedingsoplossing met procaïne-HCl hadden gestaan. Gemiddelde van 6 planten, elk met 2 bladeren.

Treatment *Behandeling*	Degree of powdery mildew development * *Mate van meeldauwontwikkeling*
control	5.0
75 p.p.m. procaine HCl	4.5
150 „ „ „	2.1
300 „ „ „	0.0

*0 = no mildew/*geen meeldauw*
5 = leaves covered with mildew/*bladeren geheel met meeldauw bedekt*

The highest concentration of procaine HCl caused a slight browning of the edge of the oldest leaf.

Procaine HCl was active against mildew development on the leaves, when added to the roots in HOAGLAND solution within 3 days after inoculation. Ad-

[1] Accepted for publication 27 Dec., 1960.

ministering it at a later date did not eradicate the powdery mildew, but prevented its spread over the rest of the leaf.

Cucumber plants could also be kept free or almost free of mildew by spraying the leaves with a 1000 p.p.m. solution on the first and second day after inoculation, or by pouring 50 ml of a 0.5% solution on the soil of a 12 cm diameter flower pot with three plants, for four successive days after inoculation.

Uptake of procaine HCl by the roots and transport to the leaves was demonstrated by paper chromatography. Three weeks old cucumber plants were placed with their roots in a 300 p.p.m. solution for 48 hours. Eighty mm^3 of the leaf juice was spotted on WHATMAN no. 3 chromatography paper and the paper developed with butanol- acetid acid - water 50 : 15 : 45 v/v as a descending solvent. Since procaine provides an orange colour after diazotation and coupling with α-naphthol (JAMINET, 1951), the paper chromatogram was sprayed with a mixture of an 1% aqueous $NaNO_2$ solution and glacial acetic acid, 10 : 1 v/v, and thereafter with a mixture of a 1% alcoholic α-naphthol solution and concentrated NH_4OH, 4 : 1 v/v. In the chromatogram of leaf juice from treated plants, an orange spot appeared with a Rf value equal to that of procaine HCl.

Spraying of procaine HCl was found to be effective against the following powdery mildews:

Erysiphe cichoracearum on cucumbers and gherkins,
Erysiphe graminis DC. on wheat,
Erysiphe polygoni DC. on beets, peas and lupins,
Sphaerotheca pannosa Lév. on roses,
Podosphaera leucotricha (Ellis & Ev.) Salm. on apples.

Phytotoxicity was observed, when frequently high concentrations of procaine HCl were sprayed with intervals of about three days: necrotic spots on leaves and fruits of apple trees (0.4% procaine) and drop of the older leaves of roses (0.8% procaine).

Since no systemic protection of procaine HCl was obtained against any other fungal or bacterial diseases tested, including rust on beans, early blight on tomatoes, cucumber scab, late blight on potatoes, downy mildew on lettuce, and halo blight on beans, it seems likely that it acts specifically on powdery mildews.

In vitro a concentration of 1000 p.p.m. was inactive against all ten fungi and bacteria tested. Even germination of conidia of *Erysiphe cichoracearum* appeared to be unaffected, either in vitro or on the leaves of plants in HOAGLAND solution, to which procaine HCl, 300 p.p.m., had been added. In the last case penetration of the fungus was observed into the epidermis, but no symptoms of the disease ever developed. So it seems that procaine HCl is only effective after it has penetrated into the plant. It is not yet known, whether it acts directly on the haustorium of the fungus, or via the metabolism of the plant.

Both constituents of procaine, namely p-aminobenzoic acid and diethylamino-ethanol were ineffective on pathogens for which procaine showed a remarkable activity.

More detailed results concerning the systemic action on powdery mildews of procaine HCl and a number of related substances will be published elsewhere.

I am indebted to IR. T. W. LEFERING, IR. G. S. ROOSJE and DRS. G. SCHOLTEN for carrying out experiments on powdery mildew on apples, roses and gherkins respectively, and to Miss Ingrid TULLENERS for valuable assistance.

Samenvatting

In een voorlopige mededeling wordt de systemische werking van procaïne-hydrochloride tegen echte meeldauw op komkommers en enkele andere gewassen vermeld.

Komkommerplanten, die met hun wortels in een voedingsoplossing gezet werden welke 300 d.p.m. procaïne-HCl bevatte, bleven vrij of nagenoeg vrij van meeldauw (tabel 1). Dit was eveneens het geval wanneer een 0,5 % procaïne-oplossing gegoten werd bij de wortels van in grond opgroeiende planten, of wanneer een 1000 d.p.m. oplossing op het blad gespoten werd (fig. 1). Transport van de wortels naar het blad werd aangetoond met behulp van papierchromatografie en een kleurreactie.

Na bladbespuiting was procaïne-HCl ook actief tegen meeldauw op augurken, tarwe, bieten, erwten, lupine, rozen en appels. Herhaaldelijk spuiten met hoge concentraties (0,4–0,8 %) veroorzaakte evenwel op diverse gewassen fytotoxische verschijnselen. Een aantal aan procaïne verwante stoffen werd bij de voortzetting der proeven betrokken.

Procaïne-HCl is vermoedelijk een specifiek op echte meeldauw werkend middel, daar het in vitro noch in vivo werkzaam was tegen alle andere getoetste planteparasieten. Tegen meeldauw werkt het pas nadat het door de plant opgenomen is; het heeft geen waarneembare invloed op de kieming der conidiën in vitro.

Reference

Jaminet, Fr., – 1951. Séparation de quelques anesthésiques locaux par chromatographie de partage sur papier. J. Pharm. Belg. 6:81.

T. Pl.-ziekten 67 (1961): 28–31

MULTIRESISTENTIE VAN DE BONESPINTMIJT *(TETRANYCHUS URTICAE)* IN AALSMEER[1]

With a summary: Multi-resistance in the two-spotted spider mite (Tetranychus urticae) *at Aalsmeer*

DOOR

W. HELLE en W. OUDSHOORN
Proefstation voor de Bloemisterij, Aalsmeer

INLEIDING

In een vroegere publikatie (HELLE, 1959) werd ingegaan op de verspreiding van resistente spintmijten in de snijbloemenkassen van Aalsmeer. Hierbij bleek dat de bonespintmijt *(Tetranychus urticae* Auct.) in de meeste kassen een resistentie ontwikkeld had voor middelen op basis van organische fosforverbindingen. Uit een recent onderzoek is echter gebleken dat in genoemde publikatie het met „*T. urticae*" aangeduide spint uit twee soorten bestaat, nl. *T. cinnabarinus* Boisdv. op anjer en *Gerbera* en *T. urticae* op roos (VAN DE BUND & HELLE, 1960).

De afgelopen twee jaar zijn de organische fosforverbindingen bij de bestrijding van beide soorten spintmijten verdrongen door gechloreerde koolwaterstoffen en spintoviciden. Daar het gevaar voor resistentie tegen deze groepen van bestrijdingsmiddelen niet denkbeeldig was, werd gedurende 1959 en 1960 de tolerantie voor acariciden van verschillende spintpopulaties bepaald.

In de zomer van 1960 bleek op een rozenbedrijf in Aalsmeer (bedrijf F) met het middel kelthane geen afdoende bestrijding meer mogelijk. Het spint van dit bedrijf werd in ons laboratorium onder geconditioneerde omstandigheden verder gekweekt op stamslaboon *(Phasaeolus vulgaris* L.). Gedurende een zestal generaties werd de gevoeligheid voor kelthane bepaald. Daar kelthane op alle stadia van *Tetranychus* werkt, werd zowel de werking tegen de eieren en larven als tegen de volwassen mijten onderzocht.

METHODIEK

a. Proeven over de werking van kelthane tegen eieren en larven

Tien volwassen wijfjes werden in een perspex spintkamertje gebracht aan de bovenzijde van een boneblad. Na twee dagen werden de kamertjes en de volwassen dieren verwijderd en het aantal eieren geteld. De bladeren met de afgezette eieren werden vervolgens gedompeld in het middel. Eén uur na het dompelen werden de behandelde bladeren met eieren van de plant verwijderd en op watten in petrischalen gelegd. De petrischalen werden gevuld met een standaardvoedingsoplossing. De schalen werden geplaatst in een vitrine bij een temperatuur van circa 25 C. Zes dagen na de behandeling met het middel werd

[1] Aangenomen voor publikatie 1 nov. 1960.

een telling uitgevoerd, waarbij onderscheid gemaakt werd tussen niet uitgekomen eieren, dode dieren en levende dieren. Hieruit werd de totale sterfte berekend. Elke bepaling werd in duplo uitgevoerd; het aantal eieren per bepaling bedroeg minimaal zeventig.

b. Proeven over de werking tegen volwassen mijten

Ook bij deze proeven werd gebruik gemaakt van perspex spintkamertjes. De boneplantjes werden gedompeld in het middel en na het opdrogen van het blad werden de vrouwelijke spintmijten van drie tot vijf dagen oud op het residu geplaatst. De dieren, tien in getal, werden gelokaliseerd in een spintkamertje. Elke bepaling bestond uit vijf herhalingen. De proefplanten werden ondergebracht in vitrines bij 25 °C onder constante belichting. Na vijf dagen werd de sterfte bepaald.

Een grote moeilijkheid bij de beoordeling van de sterfte door kelthane is, dat steeds dieren voorkomen die weinig of geen activiteit vertonen, geen eieren leggen, maar toch nog vrij lang kunnen blijven leven. Deze dieren werden door ons als „dood" geteld.

Resultaten

Het bleek dat de gevoeligheid van eieren en larven voor kelthane bij de verschillende generaties van de onderzochte stam van bedrijf F onderling geen belangrijke verschillen te zien gaf.

Fig. 1 geeft de mortilatiteits-kromme die werd verkregen uit de gegevens van drie proeven. Ter vergelijking is de gevoeligheid voor kelthane voor een normaal gevoelige stam weergegeven. Uit een vergelijking van de L.C. 50 blijkt duidelijk, dat er een groot verschil in gevoeligheid tussen beide stammen bestaat. Voor de gevoelige stam bedraagt deze 2 d.p.m. en voor de resistente 220 d.p.m.

Ook de werking van kelthane tegen de volwassen mijten toont aan, dat beide stammen in hun tolerantie voor dit middel verschillen (fig. 2). De L.C. 50 van de gevoelige stam is 16 d.p.m. tegen 300 van de resistente stam. Opvallend is dat de resistentie van de eieren duidelijk groter is dan de resistentie van de volwassen dieren.

Discussie

Voor zover wij konden nagaan is op het bedrijf F uit de groep van de gechloreerde koolwaterstoffen slechts kelthane voor de spintbestrijding gebruikt. Volgens de kweker is het aantal behandelingen niet meer dan tien geweest. De eerste bespuiting met kelthane geschiedde in 1958. In dat jaar was de gevoeligheid voor kelthane normaal (Helle, 1959).

Het is verontrustend, dat in zo'n korte tijd en met zo weinig behandelingen reeds een relatief hoge resistentiegraad werd bereikt. Dat ook hier sprake is van resistentie tegen de groep van gechloreerde koolwaterstoffen, dus een „cross-resistance", werd duidelijk door laboratoriumproeven met chloorbenzilaat; tegen dit middel werd namelijk eveneens resistentie geconstateerd.

Ook de kweker van bedrijf F voerde op ons verzoek bespuitingen uit met chloorbenzilaat, echter zonder afdoend resultaat.

In Aalsmeer is dit het eerste geval van multiresistentie van spint, nl. zowel voor organische fosforverbindingen als voor gechloreerde koolwaterstoffen.

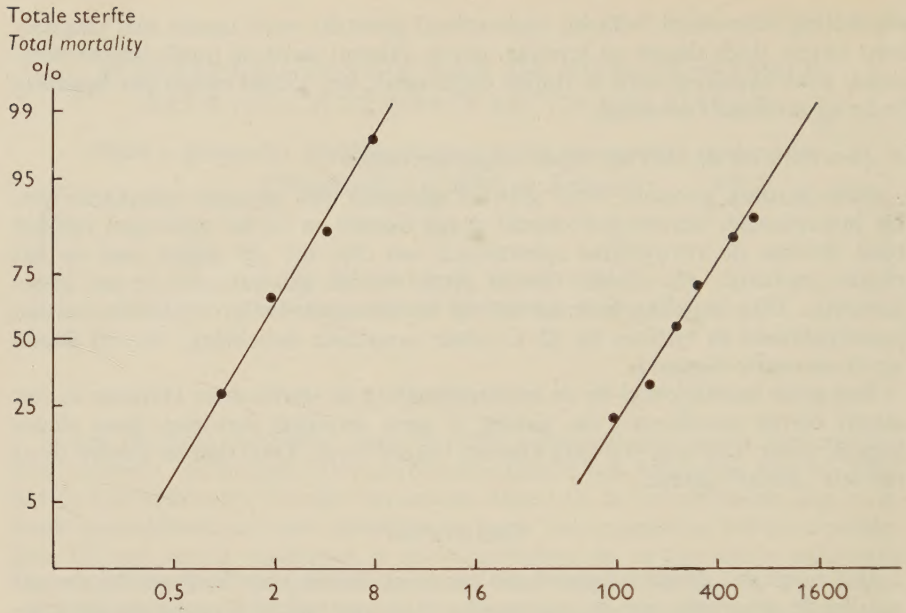


FIG. 1. Mortaliteits-kromme van de werking van kelthane 20 % m.o. tegen eieren en larven. Gemiddelde van drie proeven.

Mortality curve showing the ovo-larvicidal action of kelthane 20 % m.o. Average of three experiments.

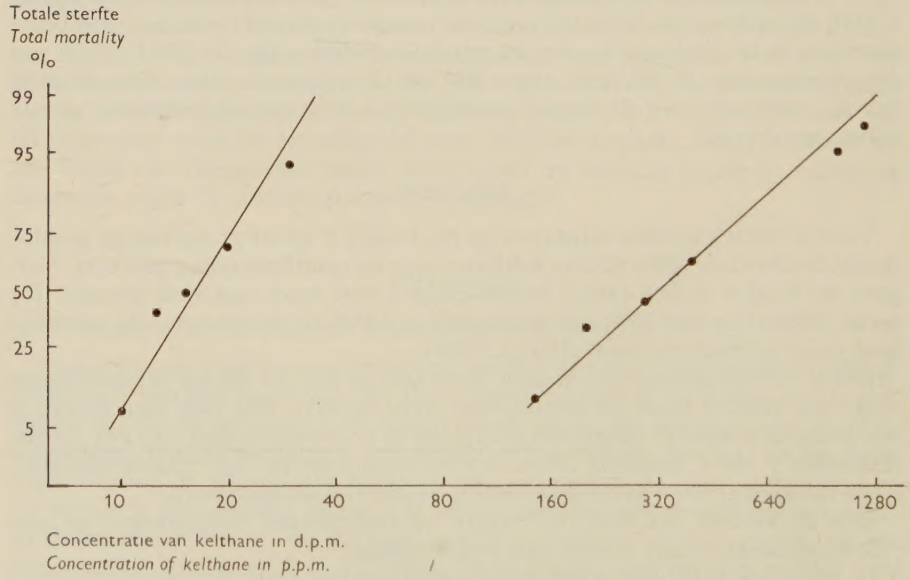


FIG. 2. Mortaliteits-kromme van de werking van kelthane 20 % m.o. tegen volwassen mijten. Gemiddelde van vier proeven.

Mortality curve showing the adulticidal action of kelthane 20 % m.o. Average of fourri expements.

Samenvatting

Een populatie van kasspint *(T. urticae* Auct.), die werd verzameld in een rozekas, werd onder laboratoriumcondities gekweekt en onderzocht op gevoeligheid voor kelthane. Het bleek, dat deze populatie resistent was tegen dit middel. Deze resistentie heeft zich binnen de tijd van twee jaar ontwikkeld. In deze twee jaar is van de groep van gechloreerde koolwaterstoffen kelthane slechts in hoogstens tien bespuitingen toegepast. Dat deze resistentie een „cross-resistance" is, wordt waarschijnlijk gemaakt door het feit dat ook voor chloorbenzilaat resistentie was opgetreden.

Summary

A spider mite population from an Aalsmeer rose-house was cultivated under laboratory conditions and its susceptibility to kelthane examined. It was found to be resistant to kelthane (figs. 1 and 2) and as it was also resistant to chlorobenzilate it must be assumed that this is a case of cross-resistance to the group of chlorinated hydrocarbons. This resistance developed within two years and after no more than ten applications. As the population was resistant to organophosphorous compounds, also, this is the first case of multi-resistance at Aalsmeer.

Literatuur

Bund, C. F. v. d. & W. Helle, – 1960. Investigations on the Tetranychus urticae complex in North West Europe. Ent. exp. & appl. 3: 142–156.

Helle, W., – 1959. Het voorkomen van resistentie tegen organische fosforverbindingen bij de bonespintmijt (Tetranychus urticae) in Aalsmeer. T. Pl.-ziekten 65: 107–115.

H. BRAUN & E. RIEHM, Krankheiten und Schädlinge der Kulturpflanzen und ihre Bekämpfung. Achte, neubearbeitete Auflage, 1957; 368 blz. met 346 fig. in de teks Uitg. Paul Parey, Berlijn en Hamburg; geheel in linnen gebonden. Prijs D.M.29.8

De zesde druk (1950) van het werk van BRAUN & RIEHM is door PROF. DR. A. J. P. OOR en DR. H. J. DE FLUITER in dit tijdschrift besproken, de zevende (1954) door DR. M. OOSTEN-BRINK. Dat er na drie jaar weer een nieuwe druk verschenen is, bewijst, dat er een grote vraa naar dit boek is.

De achtste druk wijkt in verschillende opzichten van de zevende af. De omvang is met 2 bladzijden toegenomen, het aantal figuren met 56 vermeerderd. Vele minder geslaagde afbee dingen zijn door nieuwe vervangen. Het gedeelte over de sojaboon is vervallen; verschillend nieuwe gedeelten zijn toegevoegd, nl. over virusziekten van fruitgewassen, stambasisrot (*Phy tophthora cactorum*), bessebladwesp (*Pteronidea ribesii*), Middellandse-zeevlieg (*Ceratitis capita ta*), gegroefde lapsnuittor (*Otiorrhynchus sulcatus*) en rouwvliegen (*Bibio hortulanus* en *B. marci*

Het gedeelte over de Hessische mug (*Mayetiola destructor*) is vervangen door een stuk ove *Mayetiola secalis*, welke soort in 1955 door Bollow beschreven is en door hem beschouwd wor als de Europese tegenhanger van de volgens hem alleen in de Nieuwe Wereld voorkomend Hessische mug. Volgens de mening van de huidige galmugspecialisten zijn beide soorten echt identiek, zodat de naam weer moet worden veranderd in *M. destructor*.

Uit de aard der zaak hebben de auteurs zich vanwege de uitgebreidheid van het onderwer beperkingen moeten opleggen; ze hebben slechts de voor Duitsland belangrijke cultuurg wassen opgenomen en daarvan nog weer de belangrijkste ziekten en plagen. Men kan het m hun keuze natuurlijk wel eens niet eens zijn.

Zo lijkt mij de behandeling van de in de fruitteelt zo belangrijke virusziekten op nog gee bladzijde wel wat summier. Misschien zou dit gedeelte in een volgende druk iets uitgebrei kunnen worden, temeer daar op dit gebied juist in de laatste jaren zo vele nieuwe gezicht punten naar voren zijn gekomen.

Ook de bladluizen zijn er mijns inziens wat bekaaid afgekomen. De voor de cultuurgewasse schadelijke soorten zijn in een apart gedeelte op totaal 4 bladzijden behandeld, uitgezonder de appelbloedluis (*Eriosoma lanigerum*), die onder het hoofd „Obst" in ongeveer twee en ee halve bladzijde behandeld is. De in West- en Midden-Europa minstens even belangrijke ro appelbladluis (*Dysaphis plantaginea*) en de groene appeltakluis (*Aphis pomi*) worden daare tegen niet eens genoemd.

Het aantal literatuurverwijzingen is in vergelijking met de voorgaande drukken sterk uitge breid; ze zijn in voetnoten geplaatst, wat bij de behandeling van de afzonderlijke ziekten e plagen stellig een voordeel is. Hoewel het merendeel van de opgaven nog ontleend is aan de Duitse literatuur, is ook veel aandacht geschonken aan de buitenlandse, vooral Angelsaksisch literatuur. Nederlandse artikelen worden ook vrij veel aangehaald, overwegend uit het „Tijd schrift over Planteziekten".

Het is bijna vanzelfsprekend, dat een boek als dit onnauwkeurigheden bevat. Toch zijn te veel namen van Nederlandse auteurs verminkt. Voetnoot no. 5 op blz. 287 is verwisseld m no. 1 op blz. 288.

Al met al maakt het boek toch een prettige indruk, dat ook in de toekomst zijn weg nog z vinden.

E.